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The H. K. Cushing Laboratory of Experimental Medicine

EDITED BY

G. N. STEWART, M. D., DIRECTOR

Volume V

1916-1917

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THE PREVENTION OF SIMPLE
GOITER IN MAN

BY

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THE PREVENTION OF SIMPLE GOITER IN MAN*

A SURVEY OF THE INCIDENCE AND TYPES OF THYROID ENLARGEMENTS IN THE SCHOOLGIRLS OF AKRON (OHIO), FROM THE 5TH TO THE 12TH GRADES, INCLUSIVE—THE PLAN OF PREVENTION PROPOSED.

BY DAVID MARINE, M.D., AND O. P. KIMBALL, B.S., CLEVELAND, OHIO.

SIMPLE goiter in animals is probably the easiest of all known diseases to prevent. Simple goiter includes all the thyroid enlargements seen in the lower animals and those thyroid enlargements seen in man, except cases properly classified as exophthalmic goiter. Many cases with simple goiter later develop exophthalmic goiter. In brief, simple goiter includes all those thyroid enlargements formerly classified as endemic, epidemic and sporadic. The periods when it most frequently develops are (1) fetal, (2) adolescent, and (3) during pregnancy. Anatomically a wide range of changes may be present, depending on the species of animal and on the stage (duration) of the disease. In man and fowls one more commonly sees the form characterized by an abundance of colloid material—the so-called “cystic or colloid goiter” of older writers, while in goiter of dogs, sheep, cattle, pigs, fish, etc., the accumulation of colloid material is seen only in the late, regressive or quiescent stages. Again in man the adenomatous form is very common and is exceedingly rare if present at all in the lower animals.

It will not be possible to review all the experimental data on which the assertion, that simple goiter in animals is an easily preventable disease, is based. Certain of the more important facts bearing on the subject will be summarized as an introduction to the discussion of the means proposed to attempt the prevention of simple goiter in man.

1. The developmental stage of all goiters is characterized by an increased blood flow, an increase in the size and number of epithelial cells, a decrease in the stainable colloid of the follicular spaces and a marked absolute decrease in the iodine content. The decrease in iodine precedes the cellular changes.

2. Similar thyroid changes (compensatory hyperplasia) invariably occur in the remaining portion of the gland when a sufficient portion of the entire gland is removed. The amount of gland it is necessary to remove in order to cause compensatory hyperplasia varies somewhat with the species of animal, definitely with the age, the diet, and the presence of iodine.

3. The administration of exceedingly small amounts of any salt of iodine thus far tried in any manner completely protects the remaining thyroid against compensatory hyperplasia, even after the removal of three-fourths of the normal gland in cats, dogs, rabbits and rats, fowls and pigeons. Halsted¹ and Hunnicutt² reported a series of partial thyroidectomies in dogs in which they failed to obtain the hypertrophy or hyperplasia of the remaining portion and, therefore, con-

*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.

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cluded that Halsted's earlier and justly classic experiments³ on the production of compensatory hyperplasia by partial removal were not due to thyroid removal, but to something else, possibly infection. Their failure to obtain compensatory hyperplasia in the second series was really due to the presence of available iodine either from the absorption of iodine painted on the skin or from contact with other dogs, or from inhalation of volatilized iodine from other dogs carrying iodine, or from other sources in the rooms.

4. If most of the thyroid gland is removed before or in the early stages of pregnancy and rigid steps are taken to exclude available iodine, the pups at birth will have enlarged thyroids, as first shown by Halsted,³ while if available iodine is present, the pups will have normal thyroids.⁴

5. We have repeatedly found that a milligram of iodine given at weekly intervals is sufficient to prevent thyroid enlargement, although other pups of the same litter, living in the same kennel, and eating the same food, regularly developed goiter.

6. The thyroid gland has an extraordinary affinity for iodine, as can readily be shown by perfusion experiments *in-vitro* or by injecting small amounts—5 to 20 mg. KI.—into the circulation.^{5, 6} Experimentally then the proof is sufficiently complete to demonstrate the underlying principles of goiter prevention in animals and the ease with which they can be applied. From the practical standpoint, the first instance of preventing goiter on a large scale was accidental and in connection with the sheep raising industry of Michigan. Prior to the discovery of salt deposits around the Great Lakes, the future of the industry seemed hopeless, but with the development of the salt industry and its use by the sheep growers, goiter rapidly decreased. The salt contains appreciable quantities of both bromine and iodine and in places these elements are extracted on a commercial scale. The second instance of goiter prevention on a large scale was in brook trout. Some years ago the development of goiter in artificially raised members of the salmon family became alarming and many plants were abandoned on account of the disease. After considerable work, which led to the conclusion that the disease was simple goiter, we were able to completely prevent the disease in several hatcheries, by the use of very small amounts of tincture of iodine added to the water.⁷ Later the attempt was made to substitute whole sea fish for part or all of the diet, which, likewise, proved to be, from the practical point of view, a cheaper and simpler method of complete prevention.⁸ Similar preventive work with farm stock is being carried out under our direction in some of the valleys of British Columbia, where goiter was so prevalent that farmers were unable to raise hogs, cattle, horses, and chickens on account of myxedema (cretinism). Similar work in the prevention of goiter in hogs was recently reported by Smith.⁹ He was able to completely prevent fetal myxedema by the use of potassium iodide to the mother during pregnancy. He, however, used quantities far in excess of those necessary to prevent goiter and myxedema. In spite of this knowledge of the ease and simplicity of goiter prevention in the lower animals, we know of no instance where the attempt has been made to systematically prevent or control the disease in children in large communities, especially those of the Great Lakes Basin, where goiter is so prevalent. Locally, we have been carrying out preventive treatment for the past six years at the Lakeside Hospital Medical Dispensary and have urged local

physicians to do so in their private practices. A great deal has been accomplished in this way, but as it is a public health matter the most practical and economic method would be to utilize the Public School System and the Board of Health. When the Medical Inspection of Schools is more or less independent of the Board of Health, it would be carried out through the Medical Director of Schools. This year it has been possible to begin such work on a large scale in the city of Akron, through the cooperation of the Superintendent of Schools, the Board of Education, and the County Medical Society.

It was decided for the present to limit the prophylactic work to the girl pupils, since adolescence is the most important goiter developing period and since at this period it occurs about six times more frequently in girls than in boys.

The plan now in operation was arranged from the standpoint of simplicity, practicability, economy, and the possible scientific value of the data obtained. Changes will doubtless be made as the work progresses. First a census of the condition of the thyroid gland was taken of all girls between the 5th and 12th grades inclusive and the findings recorded on individual cards, of which the following is a copy:

No.	Date	
Name	School	
Age	Weight	Physical Development
Grade	Class Standing	
Tonsils-Adenoids		
Thyroid	1	
Simple	2	
Adenomas	3	
Thyroid-tract	4	
Duration		
Remarks		

The thyroid examinations of all pupils were made by a single examiner in order to make the standards used constant and the data obtained uniform. It is planned to take the census each year in the same way.

For the prophylactic treatment we have selected sodium iodide on the grounds of economy and ease of administration. Regarding the amounts that should be given, we have no data except those from animal experimentation. As has been pointed out repeatedly, exceedingly small amounts of iodine are needed. One milligram of iodine given weekly, by mouth, is ample to prevent goiter in dogs. In all our dispensary experiments with children we have used either syrup of hydriodic acid or syrup of ferrous iodide, in 1 c.c. doses, daily for two to three weeks, repeated twice yearly, and have recommended their use to clinicians solely because they were the only U. S. P. preparations sufficiently dilute to offset the tendency to use too large amounts.

We have, therefore, arbitrarily selected to use 2 gm. sodium iodide, given in 0.2 gm. doses each school day, for each pupil in the 5th, 6th, 7th, and 8th grades; and 4 gm. given in 0.4 gm. doses each school day for each pupil in the 9th, 10th, 11th, and 12th grades. These amounts will be given twice annually about the first of May and December, at the schools by the teachers or nurses. Bottles were distributed to the several schools, containing the solutions (0.2 gm. NaI in 5 c.c. H₂O and 0.4 gm. in 5 c.c. H₂O) in sufficient amounts to give each

pupil electing to take the prophylactic treatment a total of 50 c.c. A record was made both of those who took the treatment and of those who did not. All pupils will be examined annually and the thyroid conditions recorded. These amounts of sodium iodide provide approximately 1700 (1692) mg. of iodine for each pupil of the 5th, 6th, 7th, and 8th grades and approximately 3400 (3384) mg. for the 9th, 10th, 11th, and 12th grades. When one recalls that 25 to 30 mg. saturates the normal thyroid of 20 to 25 gm. and that the thyroid has an extraordinary affinity for iodine, it seems like a prodigious waste and we believe it is. The amounts used at the start were purposely made excessive to provide for any unknown factors and will probably be materially reduced.

Analysis of the Thyroid Examinations.—Three thousand eight hundred and seventy-two girls of the 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th grades were examined and the general result is given in the following tabulation.

TABLE I.
CONDITION OF THYROID GLAND.

	NORMAL	SLIGHT ENLARGE- MENT	MODERATE ENLARGE- MENT	MARKED ENLARGE- MENT	ADENOMAS	THYROID- TRACT (PERSISTENT)
Total	1688	1931	246	7	39	594
Per cent	43.59	49.88	6.35	0.18	1.01	13.4

The thyroid glands were examined from the standpoint of *normals*, *slight*, *moderate*, and *marked enlargements*, *adenomas*, *persistent thyroglossal tracts* and the pupils for gross manifestations of *myxedema*, and *exophthalmic goiter*. No obvious case of either myxedema or exophthalmic goiter was found.

Under *normal* we have included all glands (a) which are not visible as a bulging of the skin across the trachea (b) having a barely detectable band of thyroid tissue across the trachea on palpation and (c) absence of well-defined thyroglossal stalk (so-called pyramidal process).

Those cases with enlarged thyroids have been divided into three arbitrary groups (1) *slight*, (2) *moderate* and (3) *marked* enlargement. Under *slight enlargement* we have grouped those cases with (a) visible bulging of the skin over the thyroid isthmus (except in the very stout children) and (b) a widened and thickened isthmial band or mass on palpation. If the isthmus can not be seen or felt, it can be felt by having the child swallow, while the finger or thumb is held against the trachea just below the cricoid cartilage.

Under *moderate enlargement* we have grouped those with gross deformity—bulging of the neck laterally from the enlarged lobes and marked bulging of the skin anteriorly from the enlarged isthmus. In approximately 93 per cent the right lobe was larger than the left, which is about the usual percentage.

Under *marked enlargement* we have grouped those cases with excessive deformity. One thousand six hundred and eighty-eight, or 43.59 per cent, of all pupils examined were classed as normal; 1931, or 49.88 per cent, were classed as slightly enlarged; 246, or 6.35 per cent, were classed as moderately enlarged (none of which had been operated upon); 7, or 0.18 per cent, were classed as markedly enlarged, of which two had been operated upon. This gives as totals 2184, or

56.41 per cent with enlarged thyroids and 1688, or 43.59 per cent, with normal thyroids. In 39 cases, or 1.01 per cent, adenomas, single or multiple, were detected. The smallest was approximately 2 cm. in diameter and the largest about 6 cm. These figures are of little value, since they include only the large superficial and favorably located ones.

The thyroglossal tract when present is very readily detected, either slightly to the right or left of, and rarely in, the midline. Only those which extended to the base of the thyroid cartilage were included. In many it was palpable to the hyoid bone. The very small pyramidal processes ending below the cricoid cartilage were not included. Five hundred ninety-four, or 13.4 per cent, of the cases had well-defined thyroid stalks. Physiologically the presence of thyroid tissue in the line of descent of the embryologic thyroid anlage indicates that the gland had undergone enlargement in intrauterine life, whereas normally the tract undergoes absorption beginning according to His¹⁰ in the second month. The presence of large amounts of thyroid tissue about the foramen cecum—the so-called lingual thyroid—or of large masses between the hyoid bone and thyroid cartilage—so-called infrahyoid thyroids—are of the same significance. Excluding the rare congenital defects in the thyroid anlage, the amount of thyroid tissue in the line of descent of the thyroid gland may be used to determine the degree of normality of the thyroid gland in intrauterine life and as first pointed out by Streckeisen¹¹ it is an excellent index for determining the extent and degree to which a given district is affected with simple goiter. At Basel he found about 79 per cent of the cases coming to postmortem examination had persistent thyroglossal stalks. If the district is extremely goitrous and the mothers are not

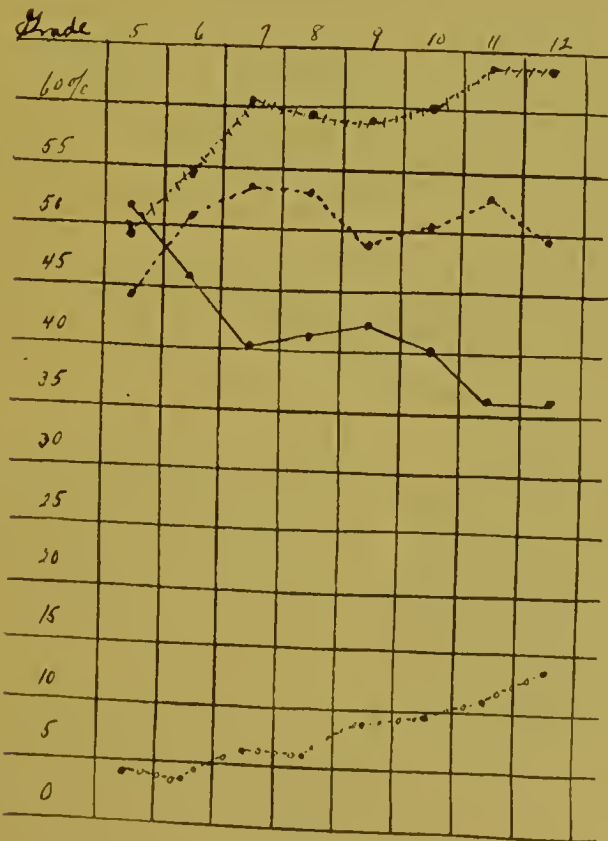


Fig. 1.

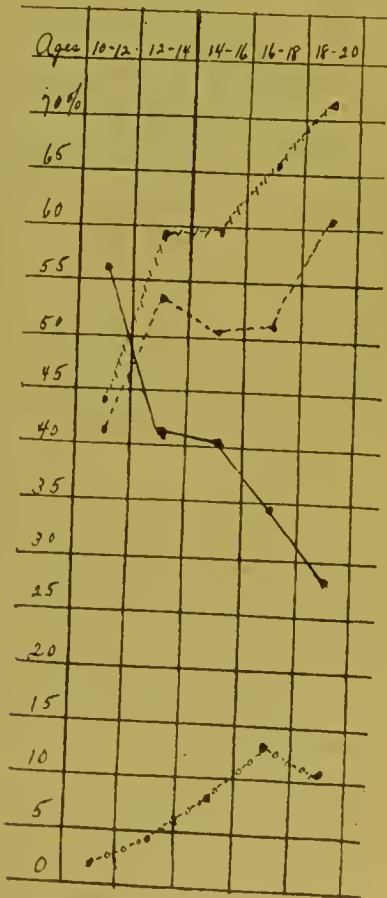


Fig. 2.

normals; - - - - slight enlargements; -o-o-o-o- moderate enlargements; -./-./-./- total enlargements.

fed iodine during pregnancy, practically all children should have large persistent thyroglossal tracts. If the district is nongoitrous (e.g., sea coast regions) very few children will have persistent thyroglossal tracts.

Following the analysis further, the condition of the thyroid in relation to grades is shown in Table II and the accompanying curve chart (see Fig. 1); and in relation to age, in Table III and accompanying curve chart (see Fig. 2).

TABLE II.
CONDITION OF THYROID ARRANGED BY GRADES.

Grades	5		6		7		8		9		10		11		12	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	410	51.77	354	45.90	269	40.0	206	40.47	191	42.92	124	40.00	76	36.02	58	36.02
Slightly Enlarged	350	44.20	388	50.33	360	53.49	271	53.24	215	48.31	155	50.00	112	53.08	80	49.70
Moderately Enlarged	31	3.90	29	3.76	43	6.39	31	6.09	38	8.54	30	9.68	23	10.90	21	13.04
Markedly Enlarged	1	0.13			1	0.14	1	0.20	1	0.23	1	0.32			2	1.24
Totals	792	20.45*	771	19.91	673	17.38	509	13.15	445	11.5	310	8.0	211	5.45	161	4.16
Adenomas**	3	0.13	3	0.13	7	0.32	6	0.28	8	0.36	6	0.28	5	0.22	1	0.04

*Percentage of total pupils examined 3872.

**Adenoma percentage figured from the total enlarged thyroids 2184.

TABLE III.
CONDITION OF THYROID ARRANGED ACCORDING TO AGES.

Age	10-12		12-14		14-16		16-18		18-20	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	530	56.08	521	41.32	460	40.35	156	34.44	21	28.77
Slightly Enlarged	394	41.69	680	53.92	578	50.70	235	51.88	44	60.27
Moderately Enlarged	21	2.22	59	4.68	98	8.6	60	13.24	8	10.96
Markedly Enlarged			1	0.08	4	0.35	2	0.44		
Totals	945	24.41*	1261	32.56	1140	29.44	453	11.70	73	1.89
Adenomas	2	0.01**	11	0.52	18	0.84	8	0.39		

*Percentage of total pupils.

**Percentage of total enlarged thyroids.

The most rapid increase in the number of slight enlargements occurs between the 5th and 8th grades. This corresponds very closely with the rapid increase, between the 10th and 14th years. The average age of the 5th grade pupils was 10 years. Less than 2 per cent of the 5th grade were under 10 years and they were tabulated in the 10 to 12 age group.

The age group 18 to 20 contains less than 2 per cent of the total pupils, and while tabulated for the sake of completeness, the percentages are doubtless higher than the normal average for this age and properly belong to a special group with lower mental activity. The relation of thyroid enlargement to retarded mental development is an important subject, but our available data do not permit of further discussion at present.

The most valuable and accurate data of the incidence of goiter in America can be obtained from examinations of the public school population, because, in the first place it covers the most important ages when goiter develops; secondly, it gives the most complete census; and thirdly, no additional expense or additional effort is necessary. Up to the present time no organized and systematic effort has been made in this country to study the incidence of goiter in the school populations of large communities, even in the Great Lakes Basin—the largest and most densely populated of all goiter districts of North America.

The report by Hall¹² of the examination of 3339 students at the University of Washington is the most extensive available in American literature. Of the 2086 men with the average age of 20 years and 5 months, he found 374, or 17.93 per cent, with enlarged thyroids; 272, or 13.03 per cent, classed as perceptible; 92, or 4.43 per cent, classed as medium; and 10, or 0.48 per cent, classed as large. Of the 1253 women, with the average age of 19 years and 3 months, he found 388, or 30.98 per cent, with enlarged thyroids; 294, or 23.45 per cent, classified as perceptible; 85, or 6.79 per cent, classified as medium; and 9, or 0.7 per cent, classified as large. These figures demonstrate clearly the prevalence of goiter in the northwestern states. The group is too selective and the ages too advanced to give an average incidence percentage, because (a) the greatest incidence occurs during puberty, (b) a certain percentage of enlargements recede below the level of clinical detectability spontaneously and (c) a small percentage would have receded because of iodine feeding.

In the Great Lakes Basin, Olsen¹³ reports the examination of 606 women and 193 men, presumably between the ages of 18 and 60, at Chicago. Among the women, he found an average of 17.87 per cent affected and among the men 6.72 per cent. The figures emphasize the frequency of thyroid enlargements, though they are very much lower than would be obtained from a similar number of examinations during the school age, on account of the factors of spontaneous or induced regression of the thyroid enlargements and of migrations from nongoitrous districts, which his figures necessarily include.

In Europe the statistics of Schittenhelm and Weichardt¹⁴ deal with the incidence of goiter in the school populations of certain districts of Bavaria, where goiter is prevalent. Using very liberal standards, they report incidences as high as 77 and 89 per cent of the school population affected.

In the Vosges mountains of eastern France and Alsace, MacAuliffe¹⁵ has recently reported the examination of 2311 children between the ages of 2 and 15 years. He found 288, or 12.5 per cent, affected. A comparison of our data with the data cited above is not possible. We use a much more rigid standard of normal, both clinically and anatomically. Anatomically, the strictly normal gland does not exceed 0.5 gm. thyroid per kilo of body weight, though many European writers, especially those in the Alpine goiter districts, allow as much as 1.0 gm. per kilo. In dogs, the normal thyroid gland does not exceed 0.3 gm. per kilo. Clinically, in the normal gland the isthmus can barely be felt, but the lateral lobes can not be felt.

The question of the production of exophthalmic goiter by the use of iodine may be mentioned briefly. Some Swiss writers, like Oswald¹⁶ take the extreme

view that iodine should never be used in goiter, because of the danger of producing exophthalmic goiter. Pineles¹⁷ and Koehler¹⁸ take the more moderate ground that iodine should be given cautiously to neurotic individuals with goiter. Our experience has led us to the conclusion that the risk of inducing manifestations of exophthalmic goiter from the use of iodine in physiologic doses is exceedingly small, even in those cases with large hyperplastic thyroids; i. e., the kind of thyroid enlargement which would permit of the most rapid formation and excretion of the iodine-containing hormone. The extent to which iodides are used in general medicine and surgery and the rarity of the development of signs of exophthalmic goiter is the best index of the danger. Iodine is usually employed in immensely large doses; 0.2 to 0.4 gm. NaI daily for 2 weeks would offer a great excess over the amounts necessary to saturate even the largest thyroids and probably much smaller amounts would suffice in man, as it has been proved to do in the lower animals.

While the danger of causing symptoms of exophthalmic goiter probably varies with the size and degree of active hyperplasia, all authors agree that the important factor in determining such symptoms lies outside the thyroid, either in the nervous system, or some gland like the adrenal, and antedates any thyroid changes. Klose¹⁹ has reported the production of exophthalmic goiter in nervous fox terrier dogs, by the injection of sodium or potassium iodide in 0.6 gm. doses per kilo. Those experiments were soon discredited by the work of Bordenhewer²⁰. No one else has suggested any danger from the use of iodides in the case of nongoitrous individuals, except the well-known acute iodism, which affects a small percentage of people, and, so far as known, is not related to thyroid activity. Cases with definite manifestations of exophthalmic goiter should not be given iodine, although there are cases (or better, stages) of the disease which are distinctly benefited by iodides.

The use of desiccated thyroid has well-known dangers after adolescence—mainly because of the large doses used. Both economically and practically, it would not be suitable for general use, as a prophylactic agent.

SUMMARY

In a complete census of the condition of the thyroid gland in the girls from the 5th to 12th grades of the school population of a large community in the Great Lakes goiter district, it was found that 1688, or 43.59 per cent had normal thyroids; 2184, or 56.41 per cent, had enlarged thyroids; and 594, or 13.4 per cent, had well-defined, persistent thyroglossal stalks. The community lies near the southern edge of the goiter district and it is suggested that communities near the lakes would show a higher incidence. The method of prophylaxis proposed is in operation.

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THE TRANSPLANTATION OF SPLENIC TISSUE INTO THE SUBCUTANEOUS FASCIA OF THE ABDOMEN IN RABBITS.

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PLATE 54.

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The reactions of certain structures of the spleen, for example the pulp cells and the endothelium of the sinuses, to many varieties of toxins have been extensively studied. On the other hand, little is known of the nature and function of the Malpighian bodies. While morphologically they resemble lymphoid tissue, the fact that this tissue rarely shows the reactions seen in other lymphoid tissues to bacterial toxins like typhoid, streptococcus, etc., may be of significance. In certain states, however, as in lymphatism, exophthalmic goiter, etc., this tissue, like the thymus, shares in the general lymphoid hyperplasia. Then too, its arrangement as an envelope about the arteries is not duplicated elsewhere in the body. The question of the relative importance of the structures in regeneration also has received little attention. Are the Malpighian bodies, the pulp cells, and the sinuses separate tissues with separate functions, or are they more interrelated functionally and morphologically than their anatomical appearances indicate? It occurred to us that transplantation, if this was possible, would throw some light on the subject of regeneration and possibly on the relative value of the tissues in this reaction. We have not been able to find any record of the transplantation of splenic tissue where the grafts were studied from these viewpoints or from the standpoints of the growth and permanence of the grafts.

TABLE I.
Transplantation of Spleen.

Rabbit No.	Sex.	Date of splenectomy.	Date of thymectomy.	Date of transplantation.		First examination.		Histological examination.
				Autotransplantation.	Homoio-transplantation.	Day.	Condition.	
1	Male.	1915 Aug. 26	1915	1915	1915 Dec. 16	56	—	Negative (infected); infiltrated with pus cells and edematous.
2	"	" 26	Nov. 29		Nov. 29	15	—	No trace of spleen transplant found.
3	Female.	" 26	" 29		Dec. 16	65	—	Negative at examination; area not removed.
4	Male.	Nov. 5	" 29		Nov 29	17	—	Negative; nearly absorbed; marked connective tissue reaction and invasion; persistence of the spleen trabeculae; no lymph or pulp tissue present.
5	"	" 5	Dec. 2		Dec. 16 " 18	67 63	— —	Complete absorption. Absorbed area with much yellow pigment in endothelial-like cells; some lymph tissue. This is clearly an atrophic spleen transplant with persistence of the endothelial elements.
6	Female.	" 5	" 2		" 2	14	+	Positive; marked connective tissue reaction and involution. Lymph and pulp tissues still present; highly vascular; in the process of absorption.
7	Male.	" 5	" 2		" 16	65	—	Complete absorption. Several areas contain large numbers of phagocytic cells engorged with blood pigment.
8	"	" 5	" 3		" 16	67	—	Complete absorption.
9	Female.	" 5	" 3		" 16	119	—	Negative at examination; area not removed. " " " " "

10	Female.	Nov. 29	Nov. 29	Nov. 29	8	+	8	+	Positive; very well developed blood supply; marked connective tissue reaction.
11	Male.	Dec. 2	Dec. 2	Dec. 2	11	+	11	+	Positive; well organized blood supply; one large trabecula; both lymph and pulp cells present; well marked connective tissue reaction.
12	"	" 3	" 3	" 3	13	+	80	-	Negative at examination; area not removed.
13	"	" 3	" 3	" 3	13	+	13	+	Positive; in the process of absorption; marked connective tissue reaction, with ingrowth into transplant area; good blood supply.
14	Female.	" 16	" 16	" 16	Dec. 16	-	67	-	Negative at examination; area not removed.
		" 16	" 16	" 16	67	++++	325	++++	Transplant 4 by 2.5 by 2 mm.; whole transplant shows usual splenic vascularity; encapsulated; typical Malpighian body formation, trabeculae, pulp, and sinus formation.
15	"	" 18	" 18	" 18	65	-	65	-	Complete absorption.

Many experiments of dislocation, often misnamed transplantation have been reported, as the experiments of Hédon¹ of pulling the organ through an abdominal wound with the blood supply intact, and suturing it into the subcutaneous tissues. Those of Lüdke,² of introducing bits of spleen into the spleens of alien species of animals, are not related to our problem, since heterotransplantation in mammals has never succeeded. The blood vessel suture experiments of Carrel³ also have no bearing on the questions suggested above.

Brief mention of our first observations with spleen transplantation was made in a previous paper.⁴ At that time only negative results with both auto- and homoio-transplantations had been obtained.

EXPERIMENTAL.

We now wish to report the end-results of a series of transplantations made more than a year ago. Twelve attempts at homoio-transplantation and six attempts at autotransplantation were made on fifteen rabbits. The more important data relating to each experiment are given in Table I.

Method.

The method employed is the same as that used by us in the transplantation of ductless gland tissues, and consists of transferring small sections of the spleen of about 2 mm. in their greatest dimension under strict aseptic precautions. Alcohol and bichloride of mercury were used instead of iodine for skin sterilization, because it is necessary to control the intake of iodine whenever the thyroid may be involved directly or indirectly in the experiment. After making a transverse abdominal skin incision about 2 cm. in length, the subcutaneous fascia is lifted with fine forceps, punctured with a cataract knife, the tissue introduced, and the fascial opening closed with a black silk ligature, which also serves to mark the site for subsequent examinations.

¹ Hédon, E., Transplantation sous-cutanée de la rate, *Compt. rend. Soc. biol.*, 1899, vi, 560.

² Lüdke, H., Ueber Milztransplantationen, *Münch. med. Woch.*, 1909, lvi, 1469.

³ Carrel, A., Remote Results of the Reimplantation of Kidney and Spleen, *J. Exp. Med.*, 1910, xii, 146.

⁴ Manley, O. T., and Marine, D., Transplantation of Ductless Glands with Reference to Permanence and Function, *J. Am. Med. Assn.*, 1916, lxvii, 260.

The spleen was completely removed at the time of transplantation in the group of autotransplants, while in all the homoiotransplants it was removed some time (from 112 to 24 days, in the majority about 40 days) before transplantation. This is probably too long a time interval to include a possible advantage to the transplant that might accrue from a splenic insufficiency, since Musser and Krumbhaar⁵ and others⁶ have shown that certain animals (dogs) usually begin to recover from the systemic effect of splenectomy, as indicated by the erythrocyte counts, in 3 to 4 weeks.

It has been definitely established that a physiological insufficiency markedly influences the growth of autotransplants of the ductless glands, but it does not influence to any extent the taking of the transplants. Likewise with homoiotransplants there is no evidence that an induced physiological insufficiency modifies the taking. Sex also does not influence the taking, growth, or rate of destruction of the transplanted tissue. The thyroid was removed in every instance but one at the time of, or shortly before transplantation without any influence on the taking or growth of the spleen grafts. Removal of the thyroid, ovaries, and spleen at one time, with immediate autotransplantation in one animal, was without effect.

Homoiotransplantation.

Direct and microscopic examinations were made in three cases on the 14th, 15th, and 17th days, while in the remaining ones it was delayed for 2 or more months. Only in one instance, the 14 day transplant, was there definite splenic tissue remaining, and in this only the lymphoid tissue and trabeculæ could be recognized. The one outstanding difference between the spleen and other tissue transplants is the marked early connective tissue reaction resembling

⁵ Musser, J. H., Jr., and Krumbhaar, E. B., The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. VI. The Blood Picture at Various Periods after Splenectomy, *J. Exp. Med.*, 1913, xviii, 487.

⁶ Sollberger, H., Beiträge zur Physiologie der Drüsen. XIX. Fortgesetzte Beiträge zur Lehre von der Funktion der Milz als Organ des Eiweissstoffwechsels. Über die Kompensationsvorgänge nach Milzexstirpation, *Biochem. Z.*, 1913, lv, 13.

the granulation tissue formation seen in chronic inflammation. Infection from organisms in the spleen, however, is not probable, as only normal spleens from rabbits with no evidence of acute infection were used. It is more probable that the splenic tissue when transferred to the subcutaneous fascia is an active irritant. The studies of Carrel⁷ on the effect of tissue extracts on the growth of connective tissue *in vitro* are of interest in this connection. He found that adult spleen extracts, thyroid extracts, and the Rous chicken sarcoma extract markedly accelerated the proliferation of connective tissue. Our observations on the irritant effect of spleen grafts confirm his observations. The effect is present in auto- as well as homoiografts, though perhaps the homoiografts excite a slightly greater connective tissue reaction. Carrel also found that thyroid extracts excite in the living animal an even more marked connective tissue proliferation both in healing skin and periosteum wounds. We have been unable to detect an excessive connective tissue proliferation around thyroid grafts. It would seem, as suggested by Carrel, that spleen extracts could be used to promote the granulation of wounds.

Autotransplantation.

Four transplants were examined on the 8th, 11th, and 13th days, respectively. All were positive and seemingly active. The 8th, 11th, and one 13th day transplants were removed for histological examination. In contrast with the homoiotransplants, examined after approximately the same period, all were positive with well established blood supplies and central necrosis, and the peripheral zone of pulp, lymphoid, and trabecular tissues was distinct. There was also the same connective tissue reaction seen in the homoiotransplants.

Of the three transplants examined at the 65th, 67th, and 80th days, complete absorption leaving large white scars at the sites had occurred in two, while in the third (Rabbit 14) a dark red, sharply circumscribed mass appeared, the size of a small wheat kernel. There were several large vessels entering it, and except for its darker color it could

⁷ Carrel, Artificial Activation of the Growth *in Vitro* of Connective Tissue, *J. Exp. Med.*, 1913, xvii, 14.

easily have been mistaken for an autothyroid transplant. We did not remove it, and it was recovered at autopsy 325 days after transplantation, the rabbit having died of acute pneumonia and pleurisy. Grossly, the transplant measured 4 by 2.5 by 2 mm., and was dark bluish red in color. It had evidently reached its maximum growth before the first examination on the 67th day, since it was not noticeably larger on the 325th day than at the first examination. This could be interpreted as evidence that its growth had occurred in response to a physiological insufficiency during the time when other tissues were assuming the function lost through removal of the spleen.

Microscopic examination reveals typical congested splenic tissue embedded in striped muscle and mammary gland. The capsule is very thick as compared with transplants of ovary, parathyroid, thyroid, or adrenal, and several connective tissue bands extend from the capsule to the surrounding muscle fascia. Many large vessels run in the outer layer of the capsule. Small trabeculae also extend throughout the gland parenchyma. Their numbers are the same that one finds in the normal spleen, while their size is proportional to the size of the organ. No attempt was made to demonstrate the presence or absence of smooth muscle fibers in the capsule and trabeculae and no studies on the reticulum have been made. In some sections one can see as many as sixteen well defined Malpighian bodies with the characteristic central artery and radial capillary system. These Malpighian bodies are surrounded by typical splenic pulp with large highly congested sinuses. In places there are deposits of the yellowish brown blood pigment usually seen in the normal spleen pulp. We have, therefore, to deal with a small newly formed spleen developed in an entirely foreign field as regards its location, nerves, and blood vessel relations. It has all the characteristics of the normal spleen in as far as these have been investigated, both as to the number of its component structures, capsule, trabeculae, Malpighian bodies, pulp, sinuses, and blood pigment, and their relation to each other. The trabeculae are proportional to the size of the organ, while all the other structures are of normal proportions, suggesting that the trabeculae play a purely mechanical part. The capsule is still relatively much thicker than that of the normal spleen,

and it seems probable that this is due to the enormous proliferation of the connective tissue which takes place around the graft very early (within 2 weeks), and also that this tends to return to relatively more normal proportions through absorption as the connective tissue becomes adjusted to its new neighbor. One of the striking facts, then, is that the regeneration involves all the structures in such a way that both their normal proportions and arrangements are wholly retained. This would suggest a fairly uniform vitality for all the component parts as well as a close functional interrelation. It may be recalled that in the adrenal the cortical cells readily survive, while the medullary cells invariably die, at least in our experience. When one recalls that in the thyroid only a narrow peripheral zone of three or four cells in thickness survives the transfer and that the whole interior portion undergoes necrosis, it is of much more significance that a fully regenerated organ should develop after transplantation of splenic tissue than in the case of such tissues as the thyroid or parathyroid which contain but a single specialized tissue.

The regeneration of Malpighian bodies is also of interest. Why does the lymphoid tissue not regenerate around the regenerated arteries running in the capsule and trabeculæ as well as in those of the interior of the lobule? The best studies on the taking of engrafted tissues indicate that the host supplies the new blood vessels to the transferred tissue rather than that it utilizes the possible surviving fragments in the graft. It is difficult to harmonize this with the known fact that the intralobular arteries of even a regenerated spleen develop this envelope of lymphoid-like cells. Either the vessels must be specific, which does not seem probable, or the lymphoid-like cells are specific in that they control and determine the blood vessel arrangement. If this is the case, we have proof that the Malpighian bodies represent a different type of lymphoid tissue, functionally as different from ordinary lymph node tissue as is thymic tissue.

The same questions must also be raised in connection with the development of the spleen sinuses as have been discussed in connection with the formation of the Malpighian bodies. The careful study of an appropriate series of these grafts will doubtless give valuable data, both as to the normal development and function of this peculiar

organ. The survival and growth of the pulp cells are more easily understood and the process is probably similar to that of other single type tissues.

The general features of the transplant are shown in the accompanying photomicrographs (Figs. 1, 2, and 3).

SUMMARY.

We have not found in the literature a report of an instance of permanent homoio- or autotransplantation of the spleen, or of the probably closely related spleno- and hemolymph glands. Spleen autotransplants with considerable difficulty as compared with thyroid, parathyroid, ovary, or adrenal cortex. This may be due to its complex anatomical structure. An instance of a permanent autotransplant has been observed. None of our attempts to homoio-transplant it were successful beyond the usual taking and persistence for 2 or 3 weeks, common to all homoigrafts. The successful permanent subcutaneous autotransplantation had all the morphological characteristics of a fully differentiated and functionally active spleen. This method of transplantation would seem to offer a means of learning more of the normal development, regeneration, and function of this complex tissue.

EXPLANATION OF PLATE 54.

FIG. 1. Photomicrograph of the 325 day spleen transplant (Rabbit 14), showing adjacent abdominal muscle, capsule, and general characteristics. $\times 20$.

FIG. 2. Photomicrograph of the same transplant, showing the structure. $\times 100$.

FIG. 3. Photomicrograph of the same transplant, showing trabeculae, pulp, and a Malpighian corpuscle. $\times 100$.

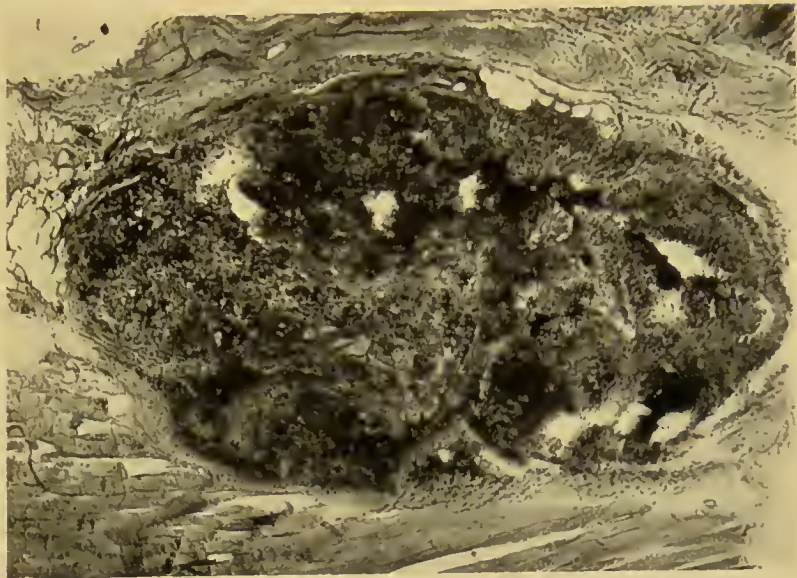


FIG. 1.

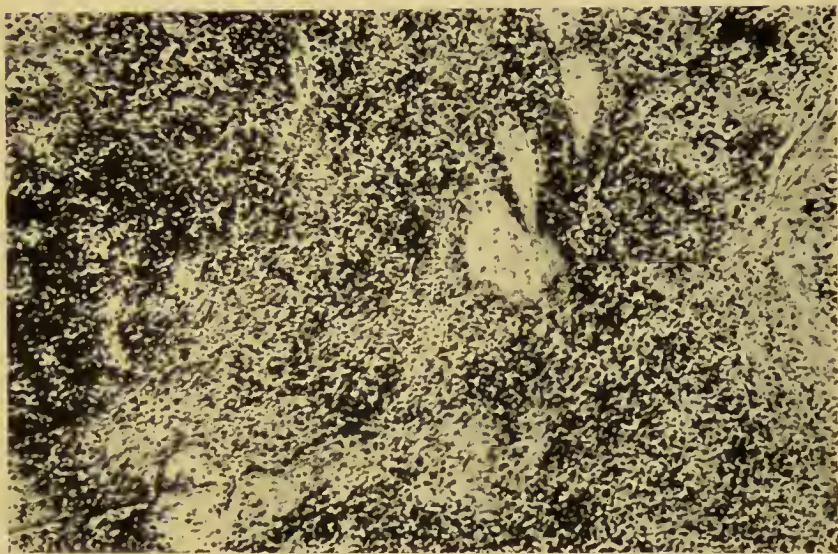


FIG. 2.

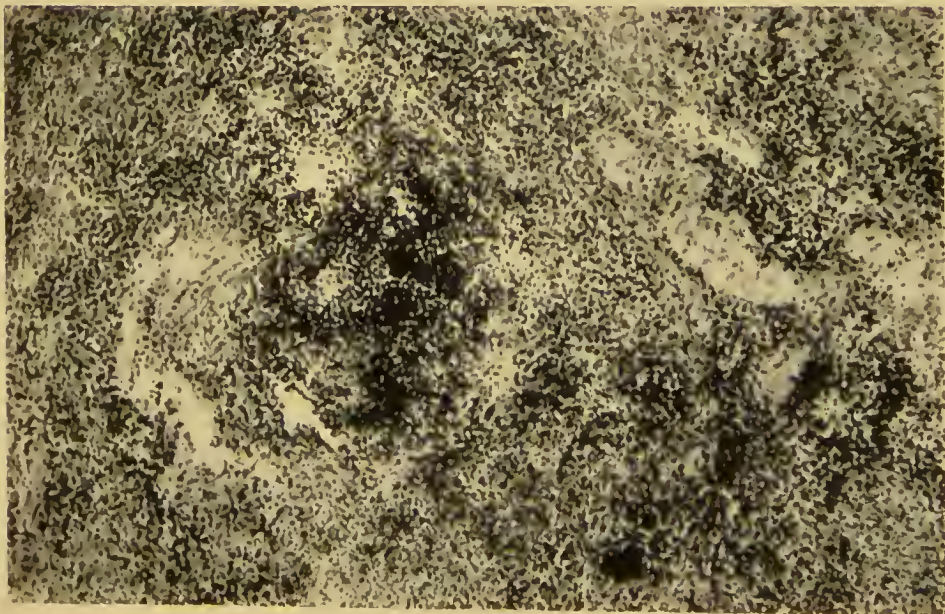


FIG. 3.

73 (1251)

**Influence of age on the permanence of subcutaneous autografts
of the spleen in rabbits.**

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We have removed the spleen and auto transplanted in 49 rabbits, varying in age from 26 days to over two years.

The method consists of introducing a small fragment of spleen, roughly 2-3 mm. in diameter, beneath the subcutaneous fascia of the abdomen, through a skin incision which is then closed by suture. These transplants have been subjected to direct examination at approximately monthly intervals to check their taking, growth or absorption. All have taken, except for three instances and these failures were due to infection. The most striking observation was the rapid growth of the grafts in the young rabbits from one to three months old and the lack of growth in the one- and two-year-old rabbits, some of which have undergone complete absorption in three months, as shown by histological examination. On the other hand, none of the transplants in rabbits less than five months old have shown any tendency to undergo absorption and histological examination shows regeneration of the major splenic elements into normal looking, encapsulated, highly vascular little spleens.

We have not been able to complete the series, with rabbits of known ages, between the period of sexual maturity (fifth month) and one year. The marked growth and activity of the transplants in young rabbits as compared with the lack of growth and tendency to absorption in old rabbits may be a part of the normal growth of the animal. In favor of this view is the fact that further growth of the transplants has not been observed after adolescence. There are no reports of differences in the systemic effects of splenectomy relative to age, although there is some

evidence from histological studies that a blood-forming function is present in early life and absent in adults. It is suggested that the age differences noted in the growth and activity of spleen autografts in addition to their probable relation to the normal growth of the animal as a whole may also be related to the loss of one of the spleen's functions in early life, through that function being assumed by another tissue.

The ease with which the spleen of young rabbits can be autotransplanted into the subcutaneous tissues might be utilized in the study of its reactions or in chemical examinations, where multiple or control spleens in accessible locations are needed.

HOW RAPIDLY DOES THE INTACT THYROID GLAND ELABORATE ITS SPECIFIC IODIN CONTAINING HORMONE?

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The thyroid offers the best opportunity of any gland for studying the rate of formation of a necessary and strictly physiological hormone because the easily detected and estimated element iodine is essential in its formation, and because it is easy to obtain glands without determinable amounts of iodine, and to cause such glands to take large amounts of iodine from the circulating blood and store it instantly (1) (2). Iodine thus taken up by the gland has been shown to have acquired no physiological activity during the first hour (1), as determined by the exceedingly sensitive biological test of Gudernatch (3),—the effect on tadpoles. It has been known for some years that striking gross and microscopic changes can be recognized in the thyroids of dogs after thirty-six hours, and also that such glands exhibit increased pharmacological activity. It seemed therefore a simple matter of obtaining a series of glands exposed to iodine for definite periods of time from one to thirty-six hours, to determine approximately the earliest appearance of increased pharmacological activity as determined by the tadpole test.

The object of this paper is to record the result obtained from the feeding of such a series of thyroids and their controls to tadpoles. The thyroid material was obtained from dogs and the experiments have been published (2) in table form in connection with a study of the rate of absorption in vivo of KI by thyroid glands. Eighteen of the thirty-three thyroids have been thus studied.

HISTOLOGIC CHANGES

Sections were taken from the control and iodized lobes of all experiments for the purpose, first, of classification, and second, of detecting the beginning of the well-known involuntary changes in the gland cells of hyperplasias of dogs' thyroids. It has many times been pointed out (4) that these changes, which consist of an increase in the stainable colloid and a shrinkage in the size of the gland cells are usually quite evident thirty-six hours after the administration of iodine in any form or manner so far studied. This series of thyroids has afforded the opportunity of studying the glands for these changes at intervals of 4, 8, 12, 16, 20, 24, and 30 hours after the intravenous injection of a constant amount (50 mgm.) of KI.

The earliest period in which definite changes were detected was in the 20-hour group (it will be recalled that the increase in thyroid iodine occurs immediately after injection), although there was a suggestion of these changes in a 16-hour experiment. These changes are necessarily more evident in the more marked hyperplasias, and as there was but one advanced hyperplasia in five 16-hour experiments, and one in four 20-hour experiments, these statements are based on but two favorable cases. Sections were made from the formalin fixed tissue after embedding in celloidin, and stained with hematoxylin and eosin. Comparing the control with the iodized lobe, there are three definite differences: (1) more stainable colloid; (2) slight shrinkage in the height of the columnar epithelium and (3) a corresponding slight increase in the size of the follicular spaces in the iodized lobe. These differences while slight, would be detected by any one familiar with a large series of dogs' thyroids as greater than one finds in untreated glands of similar time differences.

We have always assumed the occurrence of these changes indicated a physiological rather than a possible physical effect of iodine, and the discussion of the pharmacological activity of such thyroids to follow will show how closely the conclusions based on morphological changes agree with those based on pharmacological activity.

PHARMACOLOGICAL STUDIES

Tests for differences in the pharmacological activity were made after the plan used by Lenhart (5) viz.: of using white enamel dishes, each containing about 200 cc. of water and 5 tadpoles. Tap (lake) water was used and changed each morning and evening. Fifty mgm. of dessicated thyroid was added every second day and left in approximately fifteen hours. Bits of fresh liver (hog or sheep) were given on alternate days. All the tadpoles used were brought to the laboratory May 13, 1916, stored in large basins and fed daily with fresh liver and cracker meal. Three series of experiments were made. The dates of starting were May 16, May 24, and June 6.

The principal data have been arranged in the following table on the basis of the number of hours the thyroids were exposed to KI before removal from the animal.

The most striking features are the absence of any difference between the effect of control and iodized thyroids of the 4-hour "perfusions,"¹ and the very striking difference between the effect of control and iodized thyroid of experiment 16 of the 30-hour "perfusions."

In experiments 4, 25, 9, 20 and 17 the high iodine content and the high pharmacological activity of the control lobes masked any difference that might exist in the pharmacological activities of the two lobes.

The tadpole as an indicator is so sensitive that it is necessary to have control lobes of very low activity. The series includes several favorable experiments.

No definite difference between control and iodized lobes could be observed in the 4-hour perfusions. In the 8-hour group very slight differences in activity can be detected. This we interpret as evidence of the elaboration of the injected KI into the active hormone. In the 16- and 20-hour groups the iodized thyroids show well marked increases in activity.

¹ Perfusion is used in this connection because so far as iodine-thyroid effects are concerned injections of KI into the circulation are identical with in vitro perfusions of the thyroid.

EXPERIMENT NUMBER	SERIES	DATE OF BEGINNING OF FEEDING	FIRST SUGGESTIVE EFFECT. NUMBER OF DAYS AND AMOUNT OF THYROID USED				FIRST DEFINITE EFFECT. NUMBER OF DAYS AND AMOUNT OF THYROID USED				IODIN CONTENT PER GRAM DRIED		EXPOSURE OF THYROID TO KI
			Control lobe		Iodized		Control lobe		Iodized		Control lobe	Iodized	
			days	mgm.	days	mgm.	days	mgm.	days	mgm.			
4	I	May 16.....	4	100	4	100	6	150	mgm.	0.86	1.20	4	
3	I II	May 16..... May 24.....	No	difference	6	200	ten days.			trace	0.95	4	
27	I II III	May 16..... May 24..... June 6.....	No	difference	17	450	twelve days.			0.00	0.80	4	
25	I II	May 16..... May 24.....	4	100	4	100	6	150	150	3.38	4.00	8	
5	I III	May 16..... June 6.....	3	100	3	100	5	150	marked	0.18	0.62	8	
26	I II	May 16..... May 24.....			6	150			200	0.15	1.11	8	
23	I II III	May 16..... May 24..... June 6.....	10	250	6	150	8	very marked	150	0.09	1.23	12	

7	I	May 16.....	8	200	8	200	9	200	trace	0.28	12
24	II III	May 24..... June 6.....	No difference	200	6	during 12 days. 150	9	200	0.15	0.62	12
9	I II III	May 16..... May 24..... June 6.....	4 3 2	100 100 50	4 3 2	100 100 50	6 5 4	150 150 100	1.11	1.54	16
29	I II	May 16..... May 24.....	5	150	4 3	100 100	4 7	100 200	0.18	0.92	16
20	I II III	May 16..... May 24..... June 6.....	4 3 2	100 100 50	4 3 2	100 100 50	5 5 3	150 150 100	0.62	1.11	20
12	I II	May 16..... May 24.....	4 5	100 150	3 3	100 100	6 7	150 200	0.12	0.77	20

EXPERI- MENT NUM- BER	SERIES	DATE OF BEGINNING OF FEEDING	FIRST SUGGESTIVE EFFECT. NUMBER OF DAYS AND AMOUNT OF THYROID USED				FIRST DEFINITE EFFECT. NUMBER OF DAYS AND AMOUNT OF THYROID USED				IODIN CONTENT PER GRAM DRIED		EXPOSURE OF THYROID TO KI
			Control lobe		Iodized		Control lobe		Iodized		Control lobe	Iodized	
			days	mgm	days	mgm	days	mgm	days	mgm	mgm	mgm	
17	I	May 16.....	4	100	4	100	8	200	6	150	1.69	2.21	24
	II	May 24.....	2	50	3	100	3	100 marked	very marked	150			
14	I	May 16.....					8	200	6	150	0.31	1.04	24
	II	May 24.....			5	150	7	200	very marked	150			
18	I	May 16.....			4	100	10	250	8	200 marked	0.00	0.46	24
	II	May 24.....	3	100			7	200	7	200			
16	I	May 16.....			3	100			4	100	0.02	1.85	30
	II	May 24.....			2	50			very marked	100			
	III	June 6.....			2	50			very marked	100			
15	I	May 16.....			4	150			6	150 marked	0.00	0.62	30
	II	May 24.....			3	100			5	150 marked			

The most marked difference in activity is seen in experiment 16, a 30-hour "perfusion." There the iodized lobe shows a very marked effect in three to four days, while the control lobe fed in equal amounts had no detectable effect during ten days.

A fact of considerable interest is the variation noted in different dogs in the rate of formation of the active substance. These variations are independent of such gross factors as the time interval, amount of KI injected, age, sex or anatomical condition of the gland. It is suggested as a possible explanation that the variations are dependent on the amount of the mother substance available in the gland in a given period with which the iodine has to combine in order to become physiologically active.

Something possibly similar to those variations are the well known variations in the action of iodine clinically in cases with goiter—notably Basedow's disease. In numerous case reports of this disease where iodine has been given in one form or another, great variations in susceptibility to its effects have been noted. Sometimes an effect resembling that of desiccated thyroid may be seen in twenty-four to thirty hours after the oral administration of iodine while not infrequently the prolonged use of iodine has no untoward effects on metabolism although the storage of iodine in the gland is similar in the two groups. One of the authors has also seen many instances of variations in the reaction of cases of Basedow's disease and indeed of cases of simple goiter to iodine, which could not be satisfactorily ascribed to differences in the glands, in the quantity or form of iodine administered, in the quantity of iodine stored in the glands or to the stage of the disease.

The occurrence of variations in the amount of the hormone formed in dogs' thyroids under conditions of relatively constant gross factors suggests that the long known human variations may be of a similar nature and dependent on variations in the quantity of the organic nucleus with which the iodine is bound.

Another fact previously known is of interest in this connection since it is probably closely related to variations in the rate

of formation of the hormone. We refer to the presence of the two forms, *active* and *inactive*, in which the iodine is held in the gland.

Kendall (6) has shown that with alkaline hydrolysis of whole thyroid, fraction "a" contains the pharmacologically active iodine while the iodine in fraction "b" is inactive. Graham (7) has studied samples of human thyroid with high iodine contents showing much lower pharmacological activity than other samples with relatively low iodine contents. The work with the thyroid "perfusions" has demonstrated clearly that the iodine is taken up very rapidly (in a few seconds) in all cases and definitely fixed in the gland so as to withstand ordinary washing. The rate of absorption is fairly constant for glands of constant types, while as above mentioned the formation of the pharmacologically active iodine is very slow and shows wide variations in different animals. This suggests that the two processes are distinct and, as mentioned above, that the rate of activation of the iodine is controlled by the amount of the chemical nucleus or mother substance available and not by the amount of iodine, when this element is present in ordinary quantities. It is not profitable at this time to review the speculations as to the possible chemical nature of this mother substance beyond stating that it contains an aromatic nucleus and may be a tyrosine or tryptophane derivative.

In the light of the facts already pointed out a knowledge of the exact, chemical nature of this substance might give most valuable leads toward explaining certain phases of pathological physiological activity of the thyroid which have been looked for too carefully in the gland itself.

SUMMARY AND DISCUSSION

Following the injection of 50 mgm. KI into the circulation one can detect definite histological changes (always involutionary) within twenty hours in favorable cases. The more marked the hyperplasia the more readily they are detected. Definite differences in the pharmacological activity of control and iodized thyroid lobes can be detected as early as the eighth hour. This difference becomes well marked by the twentieth hour. These

facts indicate that morphological changes are closely related in time and dependent upon the elaboration of the iodine containing hormone and that the generally held view that involuntary changes in the gland are the results of a decrease in functional activity of the thyroid cells and a storage and an increase of the pharmacologically active principle—the iodine containing hormone—in the gland is essentially correct.

The storage of iodine in the thyroid from salts of this element is practically instantaneous, while the elaboration of the hormone is slow. Comparisons with the rate of formation of other physiologically necessary substances of a comparable nature are at present not possible. In the case of the suprarenal gland recent evidence (8) (9) suggests that epinephrin is probably formed more rapidly.

Whatever the rate of formation of the active substances of other ductless glands may be, it is probable that in the case of the thyroid it is relatively slow, since after thirty hours only a small fraction of the iodine taken up in as many seconds is transformed into the specific hormone.

Variations in the rate of formation of the active substance when taken in connection with the normal occurrence of iodine in the thyroid in both an active and an inactive form suggest the physiological importance of the mother substance with which the iodine is combined and the value to further work in the fields of physiology and pathology of the thyroid which a definite knowledge of its chemistry might have.

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EFFECT ON TADPOLES OF FEEDING THYROID PRODUCTS OBTAINED BY ALKALINE HYDROLYSIS

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In the following report we have recorded the results of the effects of the products of alkaline hydrolysis of the thyroid on tadpoles (larvae of *rana pipiens*). The products were prepared from normal ox, normal sheep and markedly hyperplastic lamb thyroids, after the very simple method introduced by Kendall (1) (2). He has designated the products as follows: 1, product "A;" 2, product "B;" 3, "Residue" and has described the pharmacological action of these products in man, dogs and goats. Product "A" has the typical action of desiccated thyroid of markedly accelerating metabolism. Product "B", he found to have a specific action on the skin; changing a dry scaly skin to a moist, normal condition and also relieving certain subjective symptoms of myxedema, as soreness of bones and joints and heat flashes. Basinger (3) reported the absence of any effect on the growth curve of thyroidectomized (cretin) rabbits, from the use of product "B," while product "A" had the characteristic accelerating action on metabolism noted by Kendall.

IODIN DETERMINATIONS OF PRODUCTS USED

Iodin determinations were made on the whole thyroid substance after drying and extracting with gasoline and ether. The iodine contents of the whole sheep and whole ox thyroids are approximately those usually found in normal glands (4). In contrast with the above, we obtained a batch of markedly hyperplastic and enlarged lamb thyroids, which contained no demon-

strable iodine. These three batches were hydrolyzed after the method of Kendall, and iodine determinations made on the products "A" and "B," and the "Residue." The results are given in Table I.

TABLE I

SOURCE OF THYROID	IODINE PER GRAM DESICCATED, FAT FREE	IODINE PER GRAM PRODUCT "A"	IODINE PER GRAM PRODUCT "B"	IODINE PER GRAM "RESIDUE"
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Sheep (normal).....	3.23	17.92	1.51	
Ox (normal).....	2.15	14.84	1.20	0.28
Lamb (hyperplastic).....	0.00	0.00	0.00	0.00

It is of interest to note that in the two batches containing iodine that the products contain iodine roughly in proportion to the iodine content of the whole gland. No work has been done to ascertain whether these proportions of iodine in the three products could be altered by feeding iodine, but since the iodine taken up by perfused dog thyroids is pharmacologically inactive (5) for several hours and becomes active very slowly (6), it would seem that feeding iodine must alter the proportions.

*Effect of feeding product "A"*¹

The data are given in Table II and for comparison, the effects of the corresponding whole glands are given in Table III.

Whole ox thyroid fed in 50 mgm. doses every other day caused a marked loss of weight in 2 days and definite hind legs and front leg buds were present on the 6th day. In one series, all were dead on the 13th day and in another on the 9th day. The hyperplastic lamb thyroid fed in like amount had no injurious effect, but instead caused slightly more growth than occurred in the controls, probably due to the additional food value of the thyroid.

¹ The experiments were carried out as follows: Five uniform sized tadpoles were put in each enamelware dish of about 200 cc. capacity; fed with the thyroid product every other day and fresh liver on alternate days and the water (tap) changed morning and evening of each day.

TABLE II

SOURCE OF PRODUCT	SERIES	DOSE IN MGM. EVERY SECOND DAY	DEFINITE EFFECT AP-PEARED IN DAYS	AMOUNT REQUIRED TO INDUCE 1ST EFFECT	NUMBER OF DAYS BE-FORE 1ST DEATH	NUMBER OF DAYS TO LAST DEATH	TOTAL AMOUNT USED MGM.	REMARKS
Ox thyroid.....	I	10	2(mkd)	10	7	8	40	Marked emaciation and differentiation in 6 days
Ox thyroid.....	II	7.5	2(mkd)	7.5	7	8	30	Marked emaciation and differentiation in 6 days
Ox thyroid.....	III	4	3	8	8	9	20	Marked emaciation and differentiation in 6 days
Lamb thyroids (hyperplastic)	I	10			44	50	1200	No emaciation. No differentiation
Sheep thyroid...	I	10	2(mkd)	10	8	9	40	Marked emaciation. Marked differen-tiation
Control.....		Fresh liver			41	59		

TABLE III

SOURCE OF PRODUCT	SERIES	DOSE IN MGM. GIVEN EVERY SECOND DAY	DEFINITE EFFECT IN DAYS	AMOUNT REQUIRED TO INDUCE 1ST EFFECT IN MGM.	NUMBER OF DAYS BE-FORE 1ST DEATH	NUMBER OF DAYS TO LAST DEATH	TOTAL AMOUNT USED IN MGM.	REMARKS
Ox thyroid.....	I	50	2	50	9	13	350	Legs visible on sixth day
Ox thyroid.....	II	50	2	50	7	9	250	Marked loss weight
Sheep.....								
Lamb thyroids (hyperplastic)		50	No dif-feren-tiation		25	36	850	Sacrificed. More growth than con-trols
Control.....		Fresh liver	No dif-feren-tiation		41	59		Sacrificed

Ox thyroid product "A" was fed to 3 series in 10, 7.5 and 4 mgm. doses given every other day. Definite loss of weight was noticed in 2 days, after feeding single doses of 10 and 7.5 mgm. The group getting 4 mgm. doses showed effects on the 3d day, after 8 mgm. were used.

Comparing the effect of whole ox thyroid with the effect of product "A," it is seen that 10 and 7.5 mgm. of product "A" induce more rapid and more marked emaciation and differentiation than 50 mgm. of the whole gland (see figures 1, 2, 3, 4 and 5). Four milligrams of product "A" also induced slightly more marked emaciation than 50 mgm. of the whole substance. But comparing the stages of differentiation and emaciation and the dates of first and last deaths, it would seem that the 4 mgm. is very nearly the equivalent of 50 mgm. of the whole gland as regards pharmacological activity. These effects indicate that the hydrolytic product per milligram was about 12 times as active as the whole gland, while as regards iodine content it is only about 7 times as great. Only one experiment was made with product "A" of sheep thyroid, using 10 mgm. These tadpoles showed more marked emaciation than those fed with 10 mgm. of ox thyroid product "A," although the dates of the first and the last death are practically the same in each series. The more marked activity of the sheep thyroid product "A" than of ox thyroid product "A" can be associated with its higher iodine content.

In sharp contrast with the very active products "A" of ox and sheep thyroids it is found that product "A" of the hyperplastic lamb thyroid has no activity when fed in similar doses of 10 mgm., although feeding was continued for 50 days. This was to be expected; since the whole gland showed no pharmacological activity. The color and quantity of product "A" obtained from these iodine-free lamb thyroids was approximately the same as the very active iodine-rich products, obtained from sheep and ox thyroids (see figures 1, 2, 3, 4, 5).

In addition to the above feeding experiments the three products "A" were injected intraperitoneally into guinea pigs, in single doses of 50 mgm., to test for any acute poisonous action. No symptoms of such action developed. Second injections of 50 mgm. given 33 days later likewise caused no acute reactions.



FIG. 1

FIG. 1. After 7 days feeding with iodine-free hyperplastic lamb thyroid in 50 mgm. doses, on alternate days. Normal growth similar to, and replacing control.

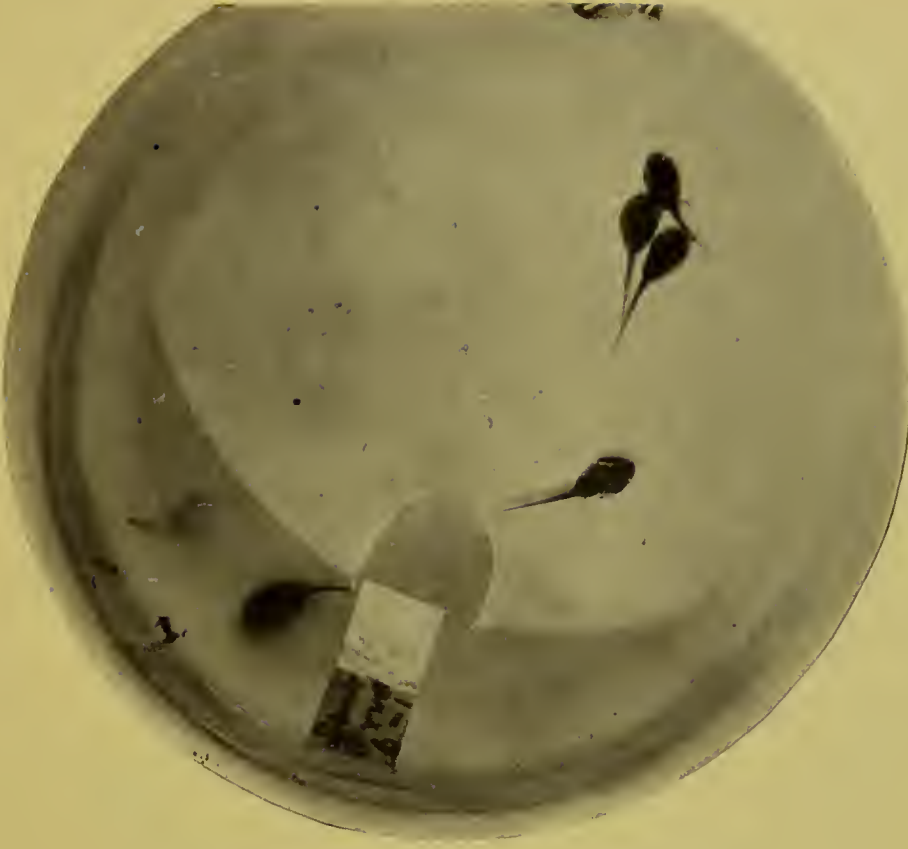


FIG. 2

FIG. 2. After 7 days feeding with iodine-free product "A" from hyperplastic lamb thyroid in 10 mgm. doses, on alternate days. Normal growth.

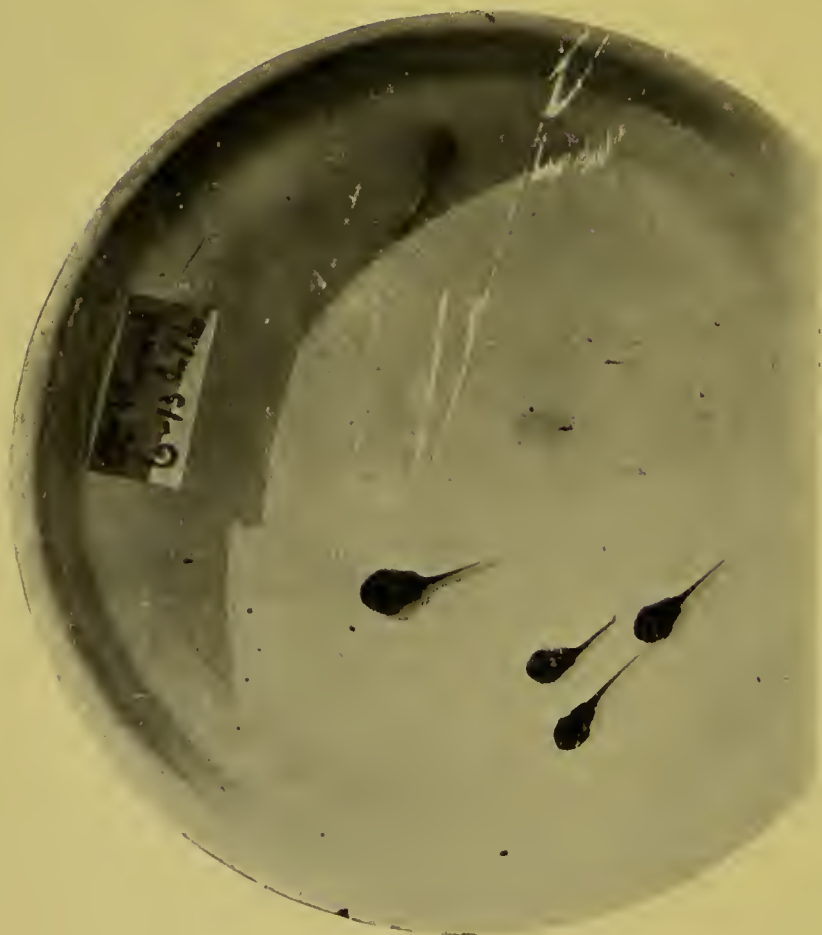


FIG. 3

FIG. 3. After 7 days feeding with ox thyroid (iodin content 2.15 mgm. per gram) in 50 mgm. doses, on alternate days. Marked loss of weight and slight differentiation.

FIG. 4. After 7 days feeding with ox thyroid product "A" (iodin content 14.84 mgm. per gram), in 10 mgm. doses on alternate days. Very marked emaciation and differentiation.

FIG. 4



EFFECT OF FEEDING PRODUCT "B"

The principal data of this series are given in Table IV. It is seen that the characteristic thyroid activity shown by the iodine-containing products "A" is absent. Product "B" is a gelatinous, hygroscopic substance, which contains iodine in much smaller

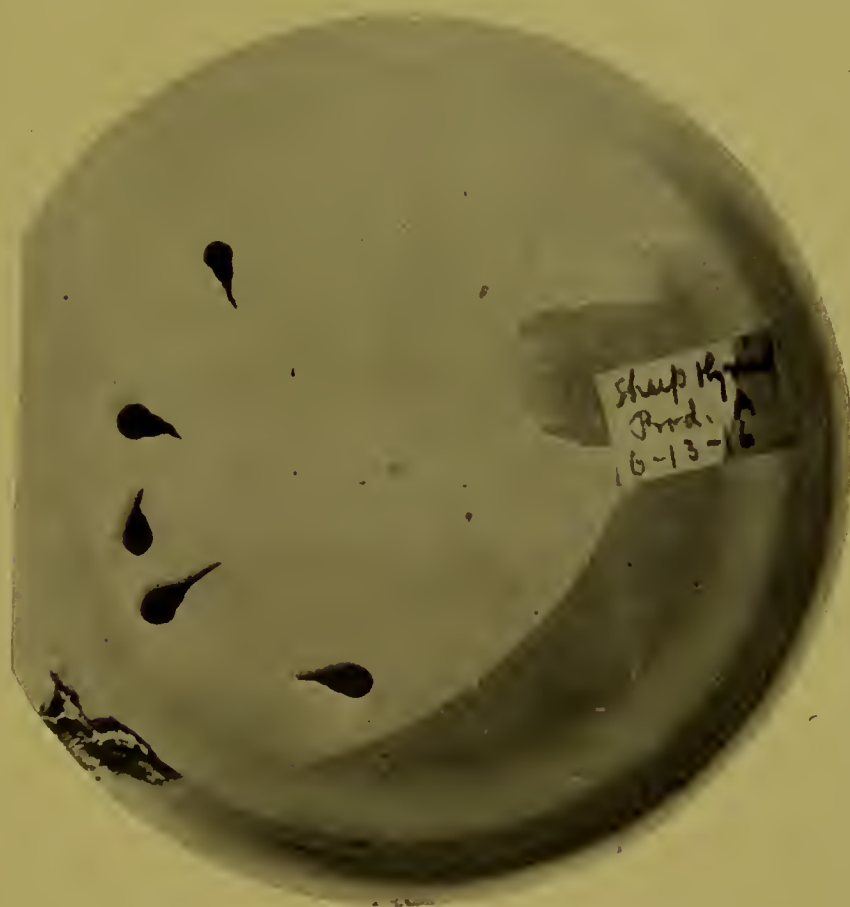


FIG. 5. After 7 days feeding with sheep thyroid product "A" (iodine content 17.92 mgm. per gram), in 10 mgm. doses, on alternate days. Extreme emaciation and differentiation.

quantities than product "A." Obviously the product "B" obtained from the iodine-free hyperplastic lamb thyroid contained no iodine.

For convenience of feeding the product "B" was dissolved in water and kept on ice. In the first series the substance was fed in 50, 100 and 150 mgm. doses, given every other day and in the

TABLE IV

SOURCE OF PRODUCT	SERIES	DOSE IN MGM. GIVEN EVERY SECOND DAY	DEFINITE EFFECT IN DAYS	AMOUNT REQUIRED TO INDUCE EFFECT IN MGM.	NUM- BER DAYS BEFORE 1ST DEATH	NUM- BER DAYS TO LAST DEATH	TOTAL AMOUNT USED IN MGM.	REMARKS
Ox thyroid.....	I	50	}	Slight loss of weight. No difference.	{ 21	36	900	Death caused by putrefaction
	II	100			19	19	1000	Death caused by putrefaction
	III	150			9	9	900	Slight loss in weight and differ-
	I	100*			14	16	1500	entiation at death.
Ox thyroid.....	II	200*	}	Loss weight, 8 days Loss weight, 8 days Loss weight and differ- entiation in 23 days	2	7	1400	Death due to putrefaction
	III	300*			1	2	600	Death due to putrefaction
	IV	400*			1	3	1200	Death due to putrefaction
	I	50			32	48	1200	Slow progressive loss of weight
Sheep.....	II	100	6		29	48	2400	and slight differentiation at
	III	150	3		29	34	2550	end of 1 month
	I	100*	3	}	10	28	2400	All show loss of weight. II
	II	200*	3		5	8	1600	and I differentiation on sixth
Sheep.....	III	300*	3		4	7	2100	day.
	IV	400*	2		2	5	2000	
	I	50	6	}	34	44	1100	All show loss of weight and
	II	100	6		25	55	2700	slight differentiation after 1
Hyperplastic lamb thyroid.....	III	150	3		41	67	4950	month
	I	100*	5		28	50	4800	All show loss of weight and
	II	200*	5		23	35	6400	slight differentiation after 1
	III	300*	2	}	2	5	1500	month
Hyperplastic lamb thyroid.....	IV	400*	2		2	4	1600	
	Fresh liver		No dif- feren- tiation		41	59		Sacrificed

* Given Product "B" daily.

second series in 100, 200, 300 and 400 mgm. doses, given every day. Each dose remained in the dishes about fifteen hours.

The experiments in which 200 mgm. or more were given daily, failed because in such amounts bacterial growth and putrefactive changes developed rapidly at room temperature. The experiments where smaller amounts were used also showed slight putrefactive changes, but not sufficient to kill the tadpoles. A slight, gradual loss of weight was noted in all the experiments and it was slightly greater in those getting 150 mgm. than in those getting 50 mgm., but this cannot be compared with the striking effects produced by the product "A" or the whole thyroid (when iodine is present—see figures 6, 7, 8, 9, 10 and 11). It seems more reasonable to account for the slight loss of weight, either on the basis that product "B" has little or no food value or on the injurious effects of the slight putrefactive changes which occurred after it was added to the water in the dishes. The iodine of product "B" cannot be responsible for the effect, since the product "B" obtained from the iodine-free hyperplastic lamb thyroids, which contained no iodine, had practically the same effect as the products which contained iodine. Comparison of the amount of loss of weight and the duration of life in the experiments where products "B" were used in smaller doses shows that the iodine-free product "B" produced a slightly smaller average loss of weight and the average life of the tadpoles was slightly longer than in the experiments with the product "B" from ox and sheep thyroids. The differences, however, are slight and might well be explained on the basis of an incomplete separation of products "A" and "B", in as much as the process is somewhat crude and it is possible that a very small amount of product "A" may be retained in the product "B."

From the evidence at hand, however, we believe that product "B," whether or not it contains iodine, has no specific action on tadpoles.



FIG. 6

FIG. 6. Control experiment for experiments with product "B" at 13 days. Liver on alternate days.



FIG. 7

FIG. 7. After 13 days feeding with ox thyroid product "B" (iodin content 1.20 mgn. per gram), in 50 mgn. doses, on alternate days. Slight loss in weight.



FIG. 8

FIG. 8. After 13 days feeding with sheep thyroid product "B" iodine content 1.51 mgm. per gram), in 50 mgm. doses, on alternate days. Slight loss in weight.

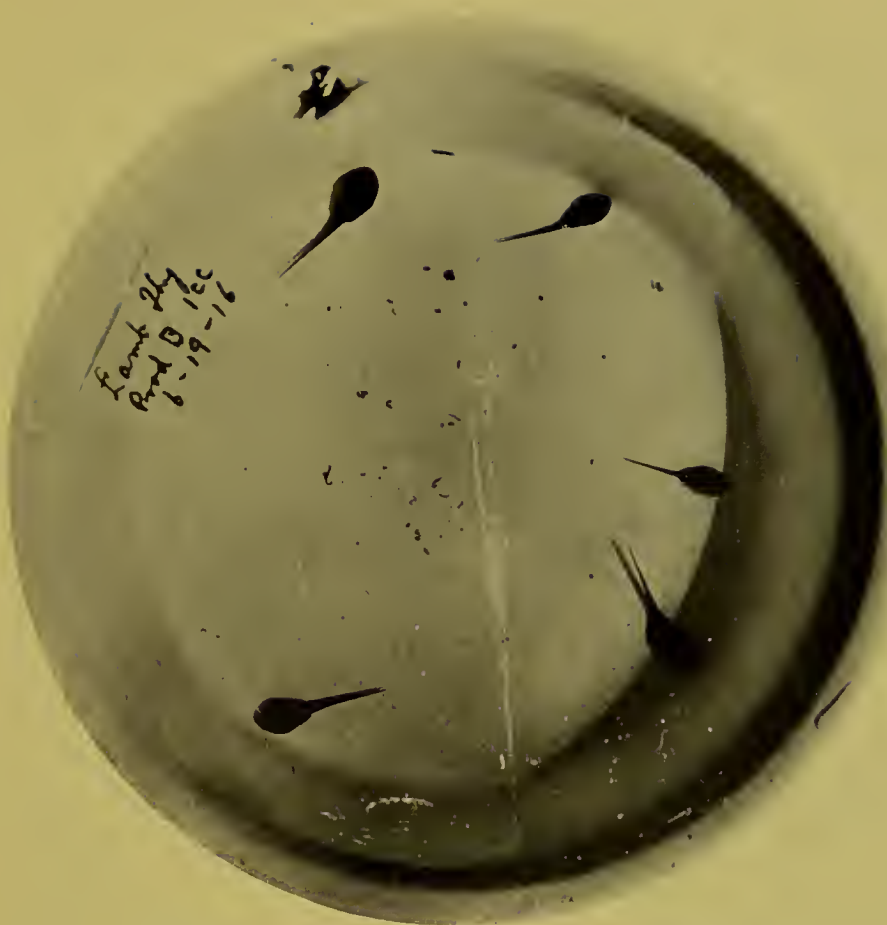


FIG. 9

FIG. 9. After 13 days feeding with hyperplastic lamb thyroid product "B" (iodine content 0.0 mgm. per gram) in 50 mgm. doses, on alternate days. Slight loss in weight.

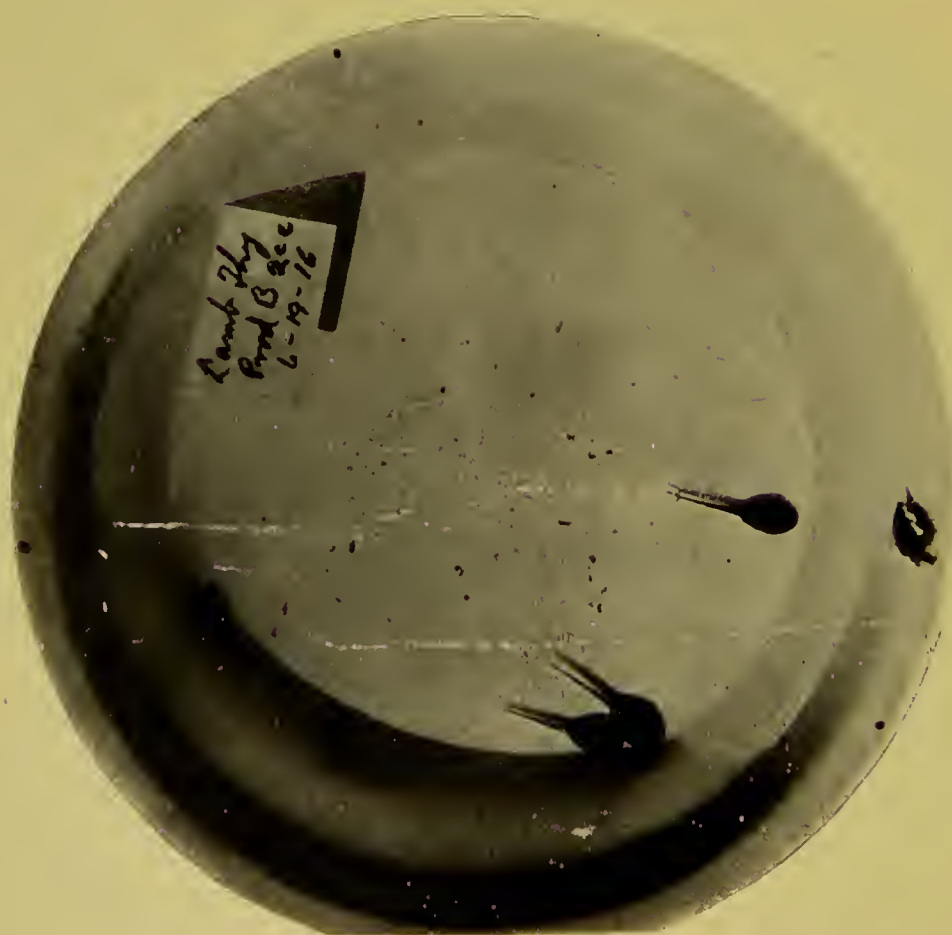


FIG. 10

FIG. 10. After 13 days feeding with hyperplastic lamb thyroid product "B" in 100 mgm. doses, on alternate days. Slight loss in weight.

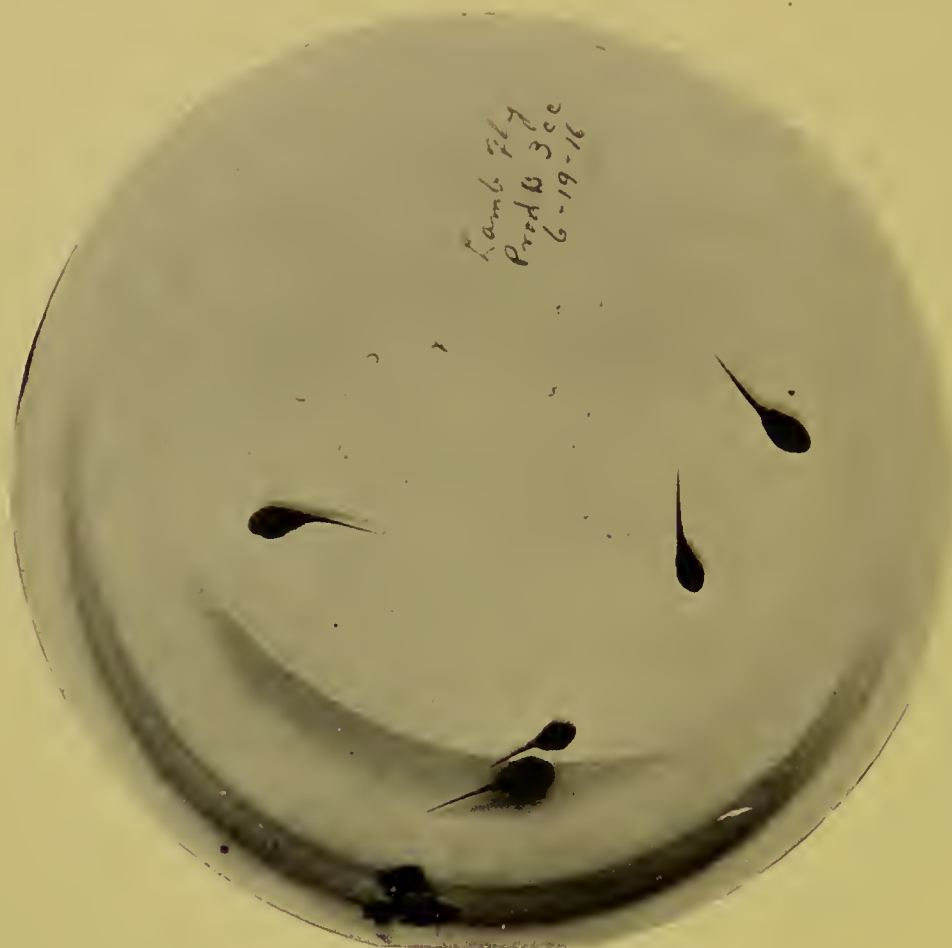


FIG. 11

FIG. 11. After 13 days feeding with hyperplastic thyroid product "B" in 150 mgm. doses, on alternate days. Slight loss in weight.

EFFECTS OF FEEDING "RESIDUE"

Experiments were made only with the "Residue" from ox thyroid. The "Residue" contained 0.28 mgm. iodine per gram. It was fed in 20 mgm. doses, given every other day. The first death took place on the 30th day and the experiment was discontinued on the 50th day, 480 mgm. of the substance having been used. Compared with the controls, no differences either in weight or differentiation could be recognized.

EFFECT OF FEEDING IODIZED PRODUCT "A" MADE FROM IODINE-FREE HYPERPLASTIC LAMB THYROIDS, AND IODIZED BLOOD PROTEIN (IODALBIN)

As was noted above, the product "A" obtained from iodine-free hyperplastic lamb thyroid does not cause the characteristic thyroid effects, although the quantity yielded and the physical characteristics were the same as those of the highly active products. It occurred to us, that possibly the "mother substance" with which the iodine must unite in order to produce the specific "hormone" was contained in the inactive product, and if so, it might be possible to activate it by artificial iodization; somewhat after the methods used for iodizing proteins, suggested by the experiments of Kurajeff (7) and others.

The iodization was carried out as follows:

The substance was dissolved in 2 per cent NaOH and warmed to 60°C. At this temperature iodine crystals were gradually added (with occasional agitation) until a slight excess was present, which was indicated by the violet color imparted to the solution, and the mixture was kept on a water bath at a temperature between 50° and 60°C. for 2 hours. It was then allowed to cool and stand for a few hours (over night). The product was then precipitated by the addition of acid (2 per cent H₂SO₄) and after filtration, the precipitate was washed with acidified (H₂SO₄) alcohol until the alcoholic filtrate no longer showed any color of iodine. The precipitate was again dissolved in alkali and then reprecipitated with acid and washed with acidified alcohol as described above. This process of dissolving in

alkali, reprecipitating with acid and washing with alcohol was repeated four times (until the alcoholic filtrate was entirely free from iodine when tested with starch solution). The precipitate was then thoroughly dried in a desiccator. The resulting product contained 52.29 mgm. iodine per gram. This substance was fed in 10 mgm. doses, given every other day; 100 mgm. being given in 22 days. No evidence of a thyroid-like effect

TABLE V
Effects of feeding Iodized Blood Protein (commercial Iodalbin)

SERIES	DOSE	DEFINITE EFFECT APPEARED IN	AMOUNT REQUIRED TO PRODUCE EF- FECT	1ST DEATH IN	LAST DEATH IN	TOTAL AM'T USED.	REMARKS
	mgm.	days	mgm.	days	days	mgm.	
I	5	6	15	22	34	85	Marked differentiation and emaciation in 11 days
I	2	6	6	20	27	26	Marked differentiation and emaciation in 11 days
I	1	11	6	24	34	17	Marked differentiation and emaciation in 16 days
II*	5	4	10	12	17	40	Complete differentiation in 12 days
II*	2	5	4	10	20	20	Complete differentiation in 13 days
II*	1	8	4	15	17	8	Emaciation and complete differentiation in 16 days
Control		gradual growth		23	Sacrificed on 39 day		

* Series II experiments were started ten days after Series I, when the stock tadpoles were larger, approaching differentiation, and during hot weather.

was produced, although the tadpoles were allowed to live for 50 days. It may be concluded that although the product has a great affinity for iodine, it does not unite with the specific organic nucleus to produce the hormone. This may be due either to the absence of the specific nucleus from this protein product "A," or though present, iodine cannot be introduced by such a procedure.

It has been shown, however, that it is possible to combine

blood protein with iodine (Kurajeff (7) and to produce a compound which has a marked influence on tadpoles. Morse (8) found that feeding "Iodalbin" to tadpoles (a commercial preparation corresponding with Kurajeff's iodized blood protein) caused effects similar to those produced by thyroid, although egg albumin iodized in the same way gave negative effects. Lenhart (9) observed some action when "Iodalbin" was fed and suggested that it might be due to the toxic effects of iodine, since this preparation has a high iodine content (about 21 per cent) in loose combination. He observed early tail absorption and emaciation, but on account of disease complications he was unable to conclude definitely that the substance had a thyroid-like action. We have made two series of experiments with "Iodalbin" and the principal data are given in Table V.

The substance was fed in 5, 2 and 1 mgm. doses on alternate days in order to prevent the acute poisonous effects when administered in larger amounts and also to make the dose correspond more closely as regards iodine content, with the doses of the thyroid products. In the above table it is seen that there is a distinct effect in both Series I and Series II. Series I being younger, reacted slightly slower than Series II to similar doses. The reactions roughly vary according to the dosage used, although there is practically no difference between the experiments where doses of 5 and 2 mgm. were used. The shortest time interval before definite effects were observed was 6 days for Series I and 4 days for Series II. Marked emaciation and differentiation occurred with all doses, the earliest instance being 11 days. In comparing these results with those obtained from feeding iodine-containing thyroid or products "A" the only difference that could be made was the more rapid effect of the thyroid products, leading to earlier emaciation, differentiation and death.

These observations confirm the reports by Lenhart and by Morse, that "Iodalbin" has a marked activity and also we can confirm Morse's statement that the effect of "Iodalbin" on tadpoles is similar to that produced by whole thyroid or its products. Since the action of "Iodalbin" on tadpoles is similar to that of

thyroid, the question arises whether the iodine complex determining the activity of "Iodalbumin" is not chemically identical with that of the thyroid. As all the evidence at present available indicates that the thyroid iodine is contained in an aromatic nucleus derived either from tryptophane or tyrosine, it is possible that in the artificial iodization of the blood proteins after the method of Kurajeff an iodine compound similar to that in the thyroid might be produced.

SUMMARY

Hydrolysis of whole thyroid after the method of Kendall, concentrates the substance producing the characteristic metabolic effect of thyroid. Kendall designates this substance product "A." The iodine contents of the products we obtained were about 6-7 times as high as those of the whole glands and the pharmacological activity approximately 12 times as great. The activity of product "A" is proportional to the iodine content. Product "A" does not produce symptoms of poisoning in guinea pigs, when introduced intraperitoneally.

It has long been known that the activity of whole thyroid is in general proportional to its iodine content and also that iodine-free thyroid is inactive and we have shown that an iodine-free product "A" is also inactive. Attempts to activate it by artificial iodization were negative. Product "B" and the "Residue," although they contain iodine are apparently inactive. The slight loss of weight of the tadpoles noted in most of the experiments might be due to incomplete hydrolysis or incomplete separation of product "A," in as much as the method is a crude one. This method of hydrolysis has afforded an additional means of establishing the fact that the thyroid normally contains both active and inactive iodine in varying amounts. Our experiments confirm the statements of Morse and of Lenhart that "Iodalbumin" has a thyroid-like action on tadpoles, but this action takes place more slowly.

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A METHOD FOR THE STANDARDIZATION OF THYROID PREPARATIONS

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The treatment of conditions due to physiological inefficiency of the thyroid gland by the administration of preparations of the gland has given the thyroid a conspicuous place as a therapeutic agent. Its value was at first frequently doubted, as a result of conflicting reports of the results obtained by its administration, but as the structure and functions of the gland were investigated it became known that the physiological efficiency of the thyroid bore an intimate relationship to its iodine content and that much of the reported uncertainty of action was due to the use of products poor in iodine. The value of this gland as a therapeutic agent has been made more certain by standardizing thyroid preparations, on the basis of their iodine content.

It is known that the iodine content of the thyroid varies with the histological structure of the gland (1) and that the physiological activity of the gland, when administered, is dependent upon the iodine which it contains in combination with its colloid (1 and 2). Marine, in 1907, visited various manufacturing plants and found that hyperplastic, as well as normal glands, were being used in the commercial preparations, and he called attention to the necessity of determining the iodine content, leading to the adoption of the iodine standardization by all leading manufacturers and in the United States Pharmacopoeia (ninth revision).

IS THE IODINE CONTENT AN ENTIRELY RELIABLE INDEX TO THE
THERAPEUTIC VALUE OF THYROID PREPARATIONS?

The iodine assay has served very well to improve the grade of physiologically-active preparations on the market and to eliminate many of the worthless inert products. But, since "inorganic iodine" does not serve the purpose in conditions where "thyroid iodine" is indicated, it is clear that the quantitative determination of iodine in a thyroid preparation can not be an entirely reliable index of its activity, for it is a simple matter to cause a hyperplastic, therapeutically worthless, specimen of thyroid to assay any amount of iodine, by simply adding iodine in any form.

The specific affinity of the thyroid for iodine is very well known and it has been shown that the hyperplastic gland is capable of absorbing large amounts of iodine, both in vivo and in vitro, and that when potassium iodide is injected into the circulation of an animal with hyperplastic thyroid glands, the iodine is absorbed by the gland practically as soon as it reaches the gland through the circulating blood (3 and 4).

If, a short while before being killed, a goiterous animal receives, in its food, a quantity of any salt of iodine, the thyroid will, of course, absorb it and on being assayed will show a good iodine content. Although, on assay, the iodine in such glands is present in sufficient quantities, it is not of value, when administered, in the sense of "thyroid iodine," as an activator of metabolism, as in the normal gland; for it has been shown that the iodine so absorbed by the thyroid requires some time (about twelve to twenty hours) before it is converted into the active "thyroid iodine" or the "iodine-containing hormone" of the thyroid (5).

In fact, the iodine of normal colloid glands is not entirely in the active form. Kendall (6), by alkaline hydrolysis, has obtained, from thyroid, three iodine-containing products, only one of which (product A) represents the essential activity of the gland. The writer, in collaboration with Dr. Marine (7) has recently confirmed that product A represents the active iodine

of the hydrolytic products of thyroid by feeding the products to tadpoles and further noted that product A, when obtained from hyperplastic (iodine-free) thyroid, was artificially iodized it did not produce the specific effect upon tadpoles although it contained a high percentage of iodine.

From the foregoing, it is evident that standardization of thyroid by determination of its iodine-content is not an entirely reliable indication of its therapeutic value and that a method of physiological assay as a supplement or substitute for the present method of iodine assay is very desirable. Such a method, to be useful, must of course be simple and practical.

Hunt (8 and 9) described a method in which mice were used as the test objects, and found that feeding of thyroid was capable of increasing their resistance to certain poisons (acetonitril). Having had no personal experience with this method, I am not in a position to offer comment on it, but since the author found that many other conditions, such as a large variety of diets, feeding of most of the glands concerned with reproduction, state of nutrition of the animal, etc., were capable of eliciting effects similar to that produced by thyroid feeding, although not as marked, it may be concluded that this reaction is not specific and is necessarily variable.

It must be considered that since the thyroid is capable of producing very marked physiological changes in an animal, it must be important to have as test objects animals whose thyroid glands are constant as regards their structure and physiological state of efficiency. Indeed, Hunt indicates that the thyroid and other iodine compounds, which he tested by this method, may act through their influence upon the animals thyroid glands. Since the thyroid gland, of all organs, is the one most subject to variations in structure and function, it seems likely that this method must yield results varying considerably with the condition of the thyroids of the test animals. However, the method has apparently given good results in the hands of its author, and his conclusions are similar to those obtained by the method employed by me.

For example, it was found by Hunt and Seidell (10) that iodine

must be in proper combination in the thyroid to be physiologically effective and that the therapeutic efficiency of the thyroid is in direct proportion to the amount of iodine in such combination.

The remarkable specific effect on tadpoles of thyroid feeding, described by Gudernatsch (11) is extremely delicate and can be easily observed.

Lenhart (12) showed that desiccated thyroid when fed to tadpoles causes effects on their growth and differentiation in proportion to the quantity fed and the amount of iodine present, and indeed, he suggests that this sensitive reaction might serve as a biological test for the activity of the thyroid, superior even to chemical methods.

As mentioned earlier in this paper, we further found with the tadpole test that not only is the effect in proportion to the amount fed and the amount of iodine present in the gland, but that only the iodine in proper combination is capable of eliciting the specific effect. This result was the basis for the elaboration of the method of standardization described in this paper.

Seven specimens of thyroid were obtained in retail prescription pharmacies, and were represented by the following products:¹ Parke, Davis and Company (tablets and capsules), Armour and Company (powder), Burroughs-Wellcome Company (tablets), Mulford and Company (tablets), and The Phospho-Albumen Company² (powder). A specimen of desiccated cattle thyroid, extracted with gasoline and ether, which was prepared in this laboratory from glands carefully selected as normal (from gross appearance) by Dr. Marine and myself, and having an iodine content of 0.215 per cent (assayed by Dr. Marine), was used as a standard. This preparation had previously been used for other investigations in this laboratory and its products of hydrolysis had been studied. This increases its reliability as

¹ The firm names are given for the purpose of identification. It must be remembered that the preparations were bought in the retail market and are therefore of uncertain age, and also that this test has not been in use by commercial firms.

² The druggist had this preparation in stock a very long time, being uncertain of its age.

a standard. The product A of this specimen of thyroid contained 1.48 per cent of iodine, product B, 0.12 per cent, and the residue 0.028 per cent. The amount of product A obtained was about one-eighth of the weight of the total amount of thyroid hydrolyzed. This indicates that in this specimen there is at least about 15 per cent of inactive iodine, since only product A represents the activity of the preparation. Indeed, product A showed an activity about twelve times as great as the thyroid from which it was obtained, although its iodine content was only seven times as great, again indicating that a substantial proportion of the thyroid iodine was inactive.

DETAILS OF METHOD

A stock of tadpoles (larvae of *Rana pipiens*) was brought to the laboratory on May 26, 1917, and placed in large basins of tap water on tables in an airy and well lighted room. The stock tadpoles were fed with fresh liver on alternate days and the water changed daily, or on hot days twice daily. From the stock were selected a number of tadpoles of uniform size and these were placed in small enamelware dishes of about 200 cc. capacity, each dish being about three-fourths full of tap water and containing five tadpoles. The same plan of feeding was carried out as was followed by Lenhart. Each series was studied in duplicate. The water in these dishes was changed twice daily, the tadpoles were fed fresh liver every other day and the thyroid (in a moderately coarse powder) was fed on the alternate days. It was found convenient to feed the liver about two or three hours before the second change of water, so that it would not remain in the dishes over night, thus preventing putrefaction in hot weather. The thyroid was fed after the second change of water and remained in the dishes until the next morning, when the water was changed and the dishes rinsed thoroughly. Along with each series were placed three dishes, each containing five tadpoles, these serving as controls. The controls were given only liver on alternating days and their water was changed twice daily. The dishes were placed in duplicates upon long tables in

F	0.2†	5	5	3	15	Angulation of head; weight	Angulation of head; loss of weight	10	5	25	Most of tails absorbed, hind legs; some emaciation Six have hind legs	100
		2	10	5	10	Slight angulation of head; slight loss of weight		14	7	14	Marked emaciation	
		30	2	1	30	Angulation of head; weight	Angulation of head; loss of weight	5	3	90	Most of tails absorbed, hind legs; marked loss in weight	
		10	3	2	20	Angulation of head; weight	Angulation of head; loss of weight	6	3	30	Hind leg buds; marked loss in weight	
		5	6	3	15	Slight angulation of head; slight loss of weight		11	6	30	Marked emaciation; two have hind legs	
F	0.05‡ in fresh gland	2	11	5	10	Slight angulation of head		14	7	14	Marked emaciation	50 to 60
		30	3	2	60	Marked angulation of head; marked loss of weight		5	3	90	Marked emaciation, tails absorbing; some have hind legs	
		10	5	3	30	Angulation of head; weight	Angulation of head; loss of weight	8	4	40	Marked emaciation and differentiation	
		5	9	5	25	Angulation of head; slight loss of weight		14	7	35	Marked emaciation, hind legs budding	
		2	13	7	14	Angulation of head; slight loss of weight		14	7	14	Increased emaciation	
G	0.136*	30	5	3	90	Angulation; loss of weight		9	5	150	Marked loss of weight; few have hind legs	40 to 50
		10	7	4	40	Slight angulation; loss of weight		12	6	60	Marked loss of weight	
		5	13	7	35	Slight loss of weight		14	7	35	Increased emaciation	
		2	14	7	14	Practically no action—growth		14	7	14	Growth	
		30	11	6	180	Slight angulation; loss of weight		14	7	210	Increased loss of weight; more angular	
H	0.17*	10	13	7	70	Slight loss of weight		14	7	70	Increased loss of weight	10 to 20
		5	14	7	35	Practically no action—growth		14	7	35	Growth	
		2	14	7	14	No action—growth		14	7	14	Growth	
		Progressive growth										
		Controls										

* I am indebted to Dr. Marine for these iodine determinations.

† Specimen labeled "containing not less than 0.2 per cent of iodine" (manufacturer's assay).

‡ Not less than 0.05 per cent in the fresh gland would correspond to about 0.2 per cent in the desiccated gland.

a large room, which was well ventilated and free from chemical fumes. The light in the room was good.

A preliminary series was observed, to form a general idea of the approximate comparative values of the different preparations. In this set, which was started on May 29, 1917, the tadpoles were fed with 60, 30, 10, 5 mgm. doses of each preparation. Having ascertained the relative effects of the various products, another series was started, on June 6, 1917, the doses being reduced to 30, 10, 5, 2 mgm., it being found that 60 mgm. was too high a dose to give comparative results, as the action produced was, in most cases, too rapid. A tabulation of the notes of this series, in condensed form, is given in table 1.

A third series, with doses of 20, 10, 5 mgm. was started on June 20, 1917, with older tadpoles and gave results closely corresponding to the second series. It was found that the best results were obtained with tadpoles somewhat older (two to four weeks) than those employed in the first series (seven to ten days). The effect was most easily demonstrated in tadpoles whose bodies were about 6 to 8 mm. in length.

The estimation of the physiological activity is easily done by observing the dose and time required for a certain effect on differentiation and growth to become manifest. It is a simple matter to determine the amount of a given preparation of thyroid necessary to produce a like effect in the same time. Physiological methods of assay cannot, at best, be as accurate as chemical methods. However, a difference of more than 10 per cent of the active iodine could be easily detected by this method and it is certainly not important to determine the physiological value of a therapeutic agent of this type to a closer degree than about 10 per cent. The relative values of the different preparations are expressed in the table with reference to the cattle thyroid preparation taken as 100.

It will be seen from the results tabulated that the effects produced by specimen G is roughly in proportion to its iodine content and also that specimen F, which contained the same amount of iodine as the active specimens, produced an effect equal to only about one-half the effect of the standard, while specimen H

showed a very low activity in comparison with its iodine content. This again emphasizes the fact that the value of thyroid is in proportion, not to its total iodine, but to the iodine in combination.

The possible objection to this method may be made that tadpoles are not available at all times of the year. Since the growth and development of the stock tadpoles can be retarded for a relatively long period, by keeping them in a cold place, it is possible to utilize nearly all of the summer months for this kind of work and it is probable also that by raising tadpoles and frogs on a large scale, it might be possible to regulate temperature and other conditions so that tadpoles could be available during a greater part of a year. At any rate, stock of thyroid accumulated in the winter would be available for assay during the proper period, since it would not change if kept with reasonable precautions.

SUMMARY

A method is described in which the specific action of thyroid upon tadpoles is used to assay the physiological value of commercial thyroid preparations.

Of seven products purchased in retail drugstores, two indicated about 20 per cent more activity than a standard preparation, two were equal in value to the standard, one somewhat more than 50 per cent of the standard, one less than 50 per cent, and one was a practically worthless preparation—being only about 10 to 20 per cent of the value of the standard.

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A STUDY OF THE PHYSIOLOGICAL ACTIVITY OF ADENOMATA OF THE THYROID GLAND, IN RELATION TO THEIR IODINE CONTENT, AS EVIDENCED BY FEEDING EXPERIMENTS ON TADPOLES.

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PLATES 38 TO 40.

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In 1912 and 1914 Gudernatsch¹ published the results of his experiments on feeding thyroid and other animal tissues to tadpoles. He concluded that thyroid has the "power to excite differentiation, but it lacks the power to cause growth." He used for these experiments fresh thyroid glands, the iodine content of which was not determined.

In 1914 Lenhart² carried out experiments along the same line as regards the thyroid, using for this purpose desiccated human, canine, sheep, and ox thyroids with iodine determinations on each specimen. He states that "the feeding of dried thyroid gland to tadpoles causes an early differentiation in proportion to the quantity fed or the percentage of iodine content of the gland used," and that thyroids with sufficiently low iodine content caused earlier differentiation but did not interfere materially with growth. "It all seems a question of dosage."

In Lenhart's work non-tumorous thyroid tissue was used. Since it has been established that the action of non-tumorous thyroid on tadpoles is dependent upon the iodine content, the following study was made to determine whether or not the so called tumors (adeno-

¹ Gudernatsch, J. F., Feeding Experiments on Tadpoles. I. The Influence of Specific Organs Given as Food on Growth and Differentiation, *Arch. Entwicklungsmechn. Organ.*, 1913, xxxv, 457; Feeding Experiments on Tadpoles. II. A Further Contribution to the Knowledge of Organs with Internal Secretion, *Am. J. Anat.*, 1913-14, xv, 431.

² Lenhart, C. H., The Influence upon Tadpoles of Feeding Desiccated Thyroid Gland in Variable Amounts and of Variable Iodine Contents, *J. Exp. Med.*, 1915, xxii, 739.

mata) of the thyroid, including carcinoma, would have the same action as non-tumorous thyroid and whether this action corresponds to the iodine content of the tumors.

For this purpose tadpoles (*Rana pipiens* and *Rana clamata*) were brought to the laboratory on May 8, 1915. They were fairly uniform in size (10 to 13 mm.) and age (estimated at about 1 week). The stock was kept in the laboratory in large granite basins and fed on fresh liver every day, the water (city tap) being changed once each day.

Thyroid Preparations.—Human thyroids were used in the experiments (Table I). Twenty-one specimens of desiccated thyroid were prepared from eighteen glands removed in Dr. Crile's clinic at Lakeside Hospital. Except in the case of No. 16 the portions of the fresh gland to be used were chopped fine, placed in a drying oven at 70°C. within 1 hour after removal, and allowed to remain in the desiccator from 4 to 6 days; they were then ground to a fine powder in a mortar and kept in sterile bottles until used. Iodine determinations were done on each specimen.³ No. 16 was prepared from the same gland as No. 15 after the latter had been fixed in formalin. This was done to determine the effect of formalin fixation on the iodine content and the action on tadpoles.

The list of thyroids includes two simple adolescent colloid goiters (Nos. 12, 15, and 16) without tumors or complications; one unclassified gland (No. 3) which probably represents a stage between simple colloid goiter and diffuse colloid adenomatous goiter; and three diffuse colloid adenomatous goiters (Nos. 1, 2, 6, and 17) in which there was a diffuse colloid adenomatous change throughout the whole gland. There were also eleven glands with well encapsulated single or multiple adenomata of the fetal series in various stages of growth and differentiation from the almost pure fetal type to the well differentiated colloid or simple adenoma. Many of these showed some or all of the secondary degenerative changes frequently occurring in these tumors; namely, edema, recent and old hemorrhage, hyaline scars, and areas of calcification and cyst formation. This group includes in the order of their increasing differentiation Nos. 21, 19, 8, 4, 13, 20, 9, 10, 18,

³ The iodine determinations were done by Dr. Marine.

TABLE I.
Desiccated Thyroid Preparations.

Thyroid No.	Specimen No.	Age.	Duration of goiter.	Clinical diagnosis.	Weight of specimen.	Pathological diagnosis.	Iodine per gm. of dried gland.
		yrs.	yrs.		gm.		mg.
1	11,714	57	20	Goiter, adenomatous.	700	Diffuse colloid adenomatous goiter.	0.22
2	11,714	57	20	" "	700	Diffuse colloid adenomatous goiter.	0.12
3	11,725	26	12	" " colloid, multiple.	735	Unclassified.	0.55
4	11,765	29	16	Goiter, adenomatous.	70	Hyperplastic intermediate adenoma.	0.69
5	11,818	30	18	" "	200	Intermediate adenoma, well differentiated.	1.31
6	11,819	55	31	" colloid.	225	Diffuse colloid adenomatous goiter.	0.43
7	11,821	34	7	" adenomatous.	173	Multiple intermediate adenoma, well differentiated.	0.85
8	11,825	47	15	" "	145	Intermediate adenoma.	0.06
9	11,836	44	14	" "	90	Multiple intermediate adenoma.	0.00
10	11,836	44	14	" "	90	Multiple intermediate adenoma.	or trace. 0.15
11	11,844	61	12	" multiple.	70	Multiple intermediate adenoma, well differentiated.	0.17
12	11,878	21	6	" colloid.	192	Colloid goiter, adolescent type.	0.58
13	11,886	47	40	" adenomatous.	14	Multiple intermediate adenoma.	1.23
14	11,898	58	1½	Adenoma.	273	Carcinoma; malignant adenoma.	0.00
15	11,904	18	2	Colloid goiter. Appendicitis.	87	Colloid goiter, adolescent type.	or trace. 1.00
16	11,904	18	2	Colloid goiter. Appendicitis.	87	Colloid goiter, adolescent type. (Formalin fixation.)	0.85
17	11,932	29	20	Colloid goiter.	710	Diffuse colloid adenomatous goiter.	0.18
18	11,933	55	12	Goiter, adenomatous.	545	Degenerating cystic intermediate adenoma.	0.07
19	11,938	31	16	" "	40	Intermediate adenoma.	0.06
20	11,939	44	⅓	" exophthalmic.	75	Multiple intermediate adenoma, well differentiated.	4.31
21	11,944	27	9	" adenomatous.	250	Fetal adenoma.	0.00
							or trace

11, 7, and 5. There was one carcinoma (No. 14) of the malignant adenoma type.

In Nos. 1 and 2, 9 and 10, and 15 and 16, two preparations were made from each of three different glands. No. 20 was diagnosed as exophthalmic goiter, and No. 13 had been treated in the clinic 1 year previously for exophthalmic goiter. The others were diagnosed as simple colloid goiter or simple adenomatous goiter before operation. The carcinoma was not diagnosed as a malignant tumor before operation.

In the preparation of the specimens of adenomata the tumorous tissue was stripped from its capsule, care being taken not to include any of the surrounding non-tumorous thyroid tissue. In the case of the diffuse colloid adenomatous goiters, individual adenomatous nodules were shelled out with their capsules and the total mass was treated as described.

EXPERIMENTAL.

Series A.—May 11 to June 10, 1915 (duration, 31 days). The tadpoles were kept on a table in the center of the room so that light and temperature conditions were the same for all. Temperatures of the room and water were recorded each day.

Five tadpoles were placed in each of twenty-one granite dishes of about 500 cc. capacity, using 300 cc. of city tap water. Four basins with five tadpoles each were used as controls. Sample tadpoles were killed in formalin at the beginning of the experiment for standards of comparison. The tadpoles for experiment were fed 50 mg. of desiccated thyroids, Nos. 1 to 21, every 2nd day, alternating with fresh liver. The controls were fed fresh liver every 2nd day. The tadpoles in this series were not so uniform in size as was desired.

For this series merely the date of death in the different dishes may be given. The action of the different specimens of thyroid was essentially the same as to emaciation, growth, and differentiation as in Series B which is to be described in detail and will serve for both.

The following tabulation in conjunction with Fig. 1 gives a fair idea as to the time of death of the tadpoles and their condition at the time of death or at the termination of the experiment when they were killed in formalin.

Thyroid No.	Iodine. mg.	Result.	Average time of death. days
20	4.31	All dead in 14 days.	12.4
5	1.31	" " " 16 "	13.8
13	1.23	" " " 21 "	12.8
15	1.00	" " " 22 "	21
16	0.85	" " " 24 "	22
7	0.85	" " " 19 "	16
4	0.69	" " " 28 "	23
12	0.58	" " " 24 "	22.4
3	0.55	Two " " 26 " ; three lived.	31
6	0.43	All " " 29 "	22.8
1	0.22	All alive at end of 31 days.	
17	0.18	One dead in 25 days; four lived.	31
11	0.17	Two " " 29 " ; three "	31
10	0.15	All alive at end of 31 days.	
2	0.12		
18	0.07		
8	0.06		
19	0.06	One dead in 30 days; four lived.	31
21	0.00	All but one, No. 14, lived 31 days.	
14	0.00		
9	0.00		

Controls. Sixteen lived 31 days; no forelegs present. Four died showing no other differentiation than slight growth of the posterior leg buds.

Definite changes were first noticed in this series on the 6th day in Nos. 20, 5, 13, 15, and 12. There was some wasting, beginning atrophy of the tail, and increased growth of the posterior leg buds. At the time of death all these, as well as Nos. 16, 6, 4, and 7, were smaller than the samples or the controls and showed much more differentiation than the controls.

As to the appearance of forelegs it is noteworthy that in the tadpoles in No. 20, four of which died in 12 days, all had visible left foreleg buds. In No. 5 all died between the 14th and 16th days and all showed foreleg buds. In No. 13 two died in 10 days with foreleg buds scarcely visible; forelegs were present on the other three. In No. 15 all died between the 20th and 22nd days; foreleg buds were present on two and doubtful on the other three.

Others in this series developed forelegs at different times corresponding closely in this and other respects with Series B. It is interesting to point out, however, that the large percentage of tadpoles living 31

days were being fed on thyroids with iodine contents of 0.22 mg. or less. It is also interesting that all these latter grew as much or more than the controls, and most of them showed a greater degree of differentiation.

Series B.—May 11 to June 28, 1915 (duration, 49 days). The procedure was the same as that in Series A, but smaller, white porcelain dishes with 150 cc. of water were used. The tadpoles in this series were quite uniform in size.

Table II and Fig. 2 give the results in this series, using the time of appearance of the first foreleg as an index to differentiation. This we think is justifiable because with the most active thyroid preparations used, and even in those tadpoles dying as early as 8 days this evidence of differentiation was present. It also offers a convenient

TABLE II.

Series B, Arranged According to Iodine Content.

Thyroid No.	Iodine content.	Time of appearance of first foreleg.	Average time of appearance of first foreleg.	Average time of death or killing.
	<i>mg.</i>	<i>days</i>	<i>days</i>	<i>days</i>
20	4.31	12	12.8	12.8
5	1.31	12	12.6	12.4
13	1.23	14	17.2	17.2
15	1.00	16	21.7	23.2
16	0.85	21	25.6	25.0
7	0.85	22	23.6	24.4
4	0.69	8	19.6	20.6
12	0.58	25	25.0	28.4
3	0.55	25	32.0	38.4
6	0.43	31	37.8	39.4
1	0.22	34	42.2	45.2
17	0.18	38	40.0	42.0
11	0.17	34	43.7	42.8
10	0.15	38	40.0	45.0
2	0.12	41	41.0	29.0
18	0.07	36	38.0	33.0
8	0.06	40	44.5	47.0
19	0.06	40	41.6	39.4
21	0.00 or trace.	40	46.4	48.2
14	0.00 " "	37	45.6	46.4
9	0.00 " "	40	43.2	45.0
Controls		45		

and useful method of comparison in determining the action of desiccated thyroid in causing differentiation of tadpoles. This is usually a fairly decisive indicator and may be readily recognized within a period of 24 hours of its occurrence.

In comparing Series A and B it is interesting to note that in Nos. 20, 5, 13, 15, 16, 7, 4, and 12, with iodine contents varying from 4.31 to 0.58 mg., all the tadpoles were dead in each series at the end of 31 days, the duration of Series A. In No. 3 (iodine content, 0.55

TABLE III.

Series B, Arranged According to Effects Observed on the 14th Day of the Experiment.

Thyroid No.	Iodine content.	Time of appearance of first foreleg.	Average time of appearance of first foreleg.	Average time of death or killing.
	mg.	days	days	days
20 5 13	4.31-1.23	12-14	12.6-17.2	12.4-17.2
15	1.00	16	21.7	23.2
16 7 4 12 3 6	0.85-0.43	21-31 (No. 4 in 8 days.)	19.6-37.8	24.4-39.4 (No. 4 in 20.6 days.)
1 17 11 10 2	0.22-0.12	34-41	40-43.7	42-45.2 (No. 2 in 29 days.)
18 8 19	0.07-0.06	36-40	38-44.5	33-47
21 14 9	Trace to 0.00	37-40	43.2-46.4	45-48.2
Controls		45		

mg.), two tadpoles in Series A and one in Series B were dead. In No. 6 (iodine content, 0.43 mg.) all were dead in Series A but only one in Series B. Below this level of iodine content (0.22 to 0.00 or trace) 91 per cent of each series were still living at the end of 31 days. It is evident then that a detailed description of the results in Series B will suffice for both within the time limits of 31 days. Series B is chosen for detailed study because of its longer duration (49 days).

The action of the desiccated thyroid was striking and consistent. On the 14th day of the experiment, before the iodine contents of the different thyroid preparations had been determined, the tadpoles were grouped according to the effect and the relative iodine contents of the different preparations predicted upon this basis. The prediction was correct with iodine contents from 4.31 down to 0.43 mg. in the order given in Table III. Below the level of 0.22 individual distinctions could not be made out with certainty, but all in this group were less affected than those receiving specimens with higher iodine contents.

Protocols.

Thyroid 20.—Changes were first observed in this dish on the 5th day; that is, after the second dose of thyroid. The change consisted of beginning kite-shaped appearance, prominence of the head, diminution in size or wasting, beginning atrophy of the tail, and increased growth of the hind leg buds. Three of these died on the 12th day and two on the 14th. All showed the characteristic changes and all at the time of death had visible left foreleg buds; all were smaller than the samples or controls. One became edematous.

Thyroid 5.—The changes, first noticed on the 5th day, were the same as in No. 20. Four died on the 12th day and one on the 14th, showing marked wasting and tail atrophy; three had a frog-shaped body and two were definitely kite-shaped. At the time of death left foreleg buds were visible on three and not observed on two; all were smaller than the samples or controls; two were somewhat edematous.

Thyroid 13.—The changes, first observed on the 6th day, were similar to those in Nos. 20 and 5. One died on the 14th day, two on the 16th, and two on the 20th. The left foreleg was present on each. One dying on the 20th day had both foreleg buds. All were smaller than the samples or controls; four were somewhat edematous.

Thyroid 15.—Changes similar to the above were observed on the 6th day. All showed marked wasting, tail atrophy, and considerable differentiation. These died on the 16th, 23rd, 24th, 25th, and 28th days, respectively. Two had left foreleg buds present at the time of death; the one dying on the 28th day had

both forelegs and the body was beginning to present the typical frog shape. One in this dish was lost.

Thyroid 16.—Changes were first observed on the 7th day. One died on the 21st day, 2 in 24 days, and 2 in 28 days. The left foreleg was present on three; not observed on two. All in this dish were quite edematous; all showed typical head changes and some were assuming frog-shaped bodies.

Thyroid 7.—Changes were first observed on the 7th day. Three died in 22 days, and two in 28 days. The left foreleg bud was present on all; all showed typical changes and were assuming frog bodies; slight edema in all.

Thyroid 4.—Slight change of shape was first observed on the 7th day. These tadpoles died in 8, 22, 23, 24, and 26 days. All were smaller than the controls; four were edematous. They showed various degrees of tail atrophy and were slightly kite-shaped. The three dying in 8, 24, and 26 days, respectively, had left foreleg buds. None were observed on the other two.

Thyroid 12.—Slight change of shape was first observed on the 7th day. One died in 26 days, two in 28, and two in 30. The first was highly edematous and showed no differentiation beyond the controls; no foreleg bud. The others were small and had frog-like bodies; the left foreleg bud was present on each; tail atrophy one-half to two-thirds.

Thyroid 3.—A slight change of shape was first observed on the 8th day. One died in 28 days, one in 36, two in 40, and one in 48. The first had a tadpole body, no tail atrophy, posterior leg buds about the same as the controls and no forelegs, and was highly edematous. The second had slight tail atrophy and the left foreleg bud through the skin; it was smaller than the control. The other three had frog-like bodies; both forelegs were present on each; tail atrophy one-third to two-thirds.

Thyroid 6.—In this dish it was doubtful whether definite changes could be made out on the 8th day. The tadpoles died in 31, 36, 39, 42, and 49 days. At the time of death all had left forelegs; the third and fourth had both forelegs, and on the fifth the right foreleg was present under the skin. Three had typical frog-shaped bodies and two were slightly frog-shaped.

Thyroid 1.—No change was observed up to the 8th day. Four died in 40, 43, 45, and 49 days, respectively. All were well differentiated frogs with almost complete atrophy of the tail. One which lived 49 days had a tadpole body and no forelegs. This is the first tadpole of the series fed on thyroid with iodine of 0.22 mg. or above which lived the 49 days of the experiment. Above this level of iodine content the tadpoles showed progressive degrees of wasting, and early differentiation in proportion to the iodine content. In none of these did the thyroid-fed tadpoles keep up with the controls as to growth (size), but of course they showed greater differentiation. Below this level of iodine content the thyroid-fed tadpoles grew as well as, and in many cases better than the controls (growth inversely proportional to the iodine content). At the same time there was a greater degree of differentiation among the low iodine-fed tadpoles as compared with the controls.

Thyroid 17.—In the frogs in this dish forelegs appeared in 38, 39, 40, 41, and 42 days. The first four developed into normal frogs with well developed and functioning fore- and hind legs and were killed in formalin on the 41st day. The last one developed into a normal frog and was killed on the 46th day.

Thyroid 11.—One died in 34 days, with beginning atrophy of the tail, slight changes about the head, and no forelegs. This one was smaller than the controls. The other four developed forelegs in 34, 39, 46, and 46 days, respectively. Two of these were killed in 42 days, and the last two died on the 48th day. All four were well developed frogs.

Thyroid 10.—Forelegs appeared in 38, 39, and 43 days on three. These developed into normal frogs; one died on the 40th day and two were killed on the 43rd and 46th days. The other two became highly edematous, showed no greater differentiation than the controls, and were killed on the 48th day.

Thyroid 2.—In this dish the tadpoles died in 11, 22, 34, 37, and 41 days. The first three maintained the tadpole body, had no atrophy of the tail, and developed no forelegs. Some of these were counted accidental deaths and not attributed to thyroid action. The fourth, dead on the 37th day, was becoming frog-shaped; no tail atrophy; no forelegs. The fifth was the only one that developed a foreleg (41st day). This one had a well developed frog-shaped body; tail atrophy about one-half.

Thyroid 18.—One of these was lost; two died in 15 and 35 days; and two lived 41 days and were killed in formalin. The first (15 days) showed little or no differentiation beyond the controls; no forelegs. The second (35 days) had well developed hind legs and was assuming a frog-shaped body; no forelegs or tail atrophy. The third and fourth developed forelegs in 36 and 40 days, respectively, and were killed on the 40th day. One of these was a well developed frog with almost complete atrophy of the tail; the other developed good fore- and hind legs, with little atrophy of the tail, and was highly edematous.

Thyroid 8.—Four developed forelegs in 40, 42, 48, and 48 days, respectively. One, which lived 49 days, had a tadpole body, no tail atrophy, and no forelegs. The first three developed into normal frogs with functioning fore- and hind legs. The fourth had both forelegs but a tadpole body and little atrophy of the tail. The fourth and fifth, both having a tadpole body, were larger than the controls.

Thyroid 19.—Two died in 28 and 35 days, respectively, without forelegs. Tail atrophy had begun, one having a tadpole body, the other becoming frog-shaped. Two developed forelegs in 40 days, became normal frogs, and were killed on the 43rd day. The last one developed forelegs on the 45th day, and when killed on the 48th day was fairly well differentiated.

Thyroid 21.—Forelegs appeared in 40, 46, 48, 49, and 49 days, respectively. The first developed into a normal frog and was killed on the 45th day. The other four lived 49 days; two of these had well developed frog-shaped bodies, tail atrophy one-third, and functioning fore- and hind legs; one had the left foreleg; and the last one had the left foreleg under the skin.

Thyroid 14.—Forelegs appeared in 37, 45, 48, 49, and 49 days, respectively. The first died in 37 days, with a tadpole body, slight changes about the head, and the left foreleg under the skin; it was about the size of the controls. The second developed into a normal frog with functioning fore- and hind legs. The third had a well developed frog body, tail atrophy one-third, and the left foreleg present. The fourth and fifth were becoming frog-shaped, had slight tail atrophy, and left forelegs under the skin.

Thyroid 9.—Four developed forelegs in 40, 40, 45, and 48 days, respectively. These were well differentiated, had functioning fore- and hind legs, and various degrees of tail atrophy. The fifth was killed on the 43rd day; highly edematous.

Controls.—There were four dishes with five tadpoles in each. These received fresh liver every 2nd day. The first one developed the left foreleg on the 45th day and died on the 48th day, a well differentiated frog. On the 49th day there were four dead, one with well developed hind legs and a left foreleg, also beginning tail atrophy; one with well developed hind legs and no forelegs; the other two were well preserved tadpoles with short posterior leg buds, no forelegs, and no tail atrophy. Fifteen lived for 49 days and were well preserved tadpoles.

Fig. 2 shows the condition of the tadpoles in this series at the time of death or termination of the experiment when they were killed in formalin. The different groups are arranged according to the decreasing iodine content with the controls and samples last. The individuals of each dish are arranged from left to right according to the time of death; *e.g.*, in No. 20 the first three from left to right died in 12 days and the fourth and fifth died in 14 days.

We have no satisfactory explanation for the peculiar edematous appearance of some of the tadpoles. This was observed in individuals in different dishes without regard to the iodine content and appeared in some of the controls.

Series C and D were for the purpose of determining the action of desiccated thyroid on a different variety of tadpoles and at varying ages of this species (*Rana catesbiana*).

Series C.—May 25 to June 10, 1915 (duration, 17 days). (Fig. 3.)

Tadpoles averaging 4 cm. in length were used in this series. Three were killed in formalin at the beginning of the experiment for comparison; three were used as controls and fed fresh liver every 2nd day; the experimental tadpoles were fed 50 mg. of Thyroids 20, 15, 12, 17, and 14 with iodine contents of 4.31, 1.0, 0.58, 0.18, and 0.0, or trace, every 2nd day, alternating with fresh liver. At the beginning of the experiment the tadpoles had an average length of 4 cm., just visible posterior leg buds, tadpole bodies, and no sign of forelegs.

The controls all lived 17 days and were killed in formalin. They had an aver-

age length of 4.3 cm., maintained the tadpole body, and showed slight increase in the posterior leg buds as compared with the samples.

Thyroid 14.—All lived 17 days and were killed in formalin, had an average length of 4.2 cm., tadpole bodies, slightly better growth of the posterior leg buds than the controls, and no forelegs.

Thyroid 17.—All these were lost.

Thyroid 12.—One was lost. One died in 12 days, showing marked wasting and tail atrophy; considerable growth of the hind legs; left foreleg bud present; beginning frog-shaped body; length 2.5 cm. The third lived 17 days and was killed in formalin. This one was 3 cm. in length; the body was becoming frog-shaped; left foreleg bud present; tail atrophy about one-third.

Thyroid 15.—Two died in 11 days. Each measured 2.3 cm.; there was marked atrophy of the tail; posterior leg buds 5 mm. long; left foreleg bud present on one and not on the other; heads frog-shaped. The third died in 12 days; length 2.2 cm.; left foreleg present.

Thyroid 20.—All died in 10 days, measuring 2, 2.2, and 2.2 cm., respectively. All showed marked tail atrophy; left foreleg present on each; bodies becoming fairly frog-like.

This series shows that there is no qualitative difference in the action of desiccated thyroid on the different varieties of tadpoles used.

Series D.—May 27 to June 7, 1915 (duration, 12 days). (Fig. 4.)

For this experiment two sets of tadpoles of different ages with three in each set were used. The individuals of each age were alike as to size and condition of development. The older ones averaged 8.5 cm. in length, had posterior leg buds slightly over 2 cm. long, and had no forelegs. The younger set averaged 6.5 cm. in length, had just visible posterior leg buds and no foreleg buds. One of each age was killed in formalin at the beginning of the experiment for comparison. Another of each age was used for control and fed fresh liver every 2nd day. A third one of each age was fed 50 mg. of Thyroid 20 every 2nd day, alternating with fresh liver.

Both forelegs were present on the older thyroid-fed tadpole on the 7th day; the left foreleg appeared on the corresponding control on the 9th day. There was marked atrophy of the tail of the thyroid-fed tadpoles. The younger control had no forelegs; and the posterior leg buds were 8 mm. long; it maintained a tadpole body. The younger thyroid-fed animal had a frog-shaped head and body; left foreleg present; posterior legs 15 mm. long. The older tadpoles at the end of the experiment showed about the same amount of development of fore- and hind legs on both the controls and the thyroid-fed animal. There was marked tail atrophy in the latter and practically none in the former. The older thyroid-fed tadpole had become a well developed frog, while the corresponding control had the same characteristics to a slighter degree.

Comparison of samples, controls, and Thyroid 20 in Series C and D shows that desiccated thyroid of high iodine content administered to tadpoles of the same variety at different ages and stages of development produces the same effect; namely, immediate cessation of growth, rapid metamorphosis as evidenced by atrophy of the tail, increased growth of the posterior legs, and the development of forelegs. The younger the tadpoles at the beginning of thyroid feeding, the smaller the metamorphosed frog, and *vice versa*.

DISCUSSION AND CONCLUSIONS.

It seems evident from the foregoing experiments that the so called tumors (adenomata) of the thyroid possess the property of taking up iodine and metabolizing it into the active combination in the same way that the non-tumorous thyroid tissue does, although not so readily nor to the same degree, and the action on tadpoles of feeding desiccated tumorous thyroid tissue does not differ qualitatively from feeding desiccated non-tumorous thyroid tissue. The action in either case depends upon the iodine (active iodine) content, and in the case of the adenomata bears no constant relation to the state of their growth or differentiation.

Examination of Tables II and III shows that in the main this is true. There are, however, certain discrepancies as to time of death, appearance of first forelegs, degree of emaciation, and rate of growth in certain dishes of the series, the action being not quite parallel to the iodine content. Some of these discrepancies may be explained in part by accidents of feeding, slight differences in size, age, and susceptibility of the different tadpoles receiving the same thyroid, and also by the variations in the amount of thyroid consumed by the different individuals in the same dish. Lenhart has shown that the action of the same thyroid varies with the quantity fed. Another important factor which has to be considered is the condition of the iodine itself. It was suspected at the time of these experiments that the iodine might be present in an active and an inactive form, but no satisfactory proof of this assumption, at the beginning of these experiments, was at hand. Support of this point has been afforded by the work of Kendall on the isolation of the active principle of thyroid and the separation of the iodine into two fractions. Since the com-

pletion of our experiments Marine⁴ has demonstrated by means of perfusion experiments *in vivo* and *in vitro* that iodine is rapidly taken up by the thyroid cells, and though the iodine increase in the perfused lobe may be 1,000 per cent in 2 hours as compared with the control lobe, yet the action on tadpoles is no greater. It then becomes an important question to determine the time required by the thyroid to take up inorganic iodine and manufacture it into the active thyroid principle.

It is known that iodine is rapidly taken up by the thyroid, and in man the iodine content of the thyroid is subject to greater variations than in animals on account of the prevalent therapeutic use of iodine and the iodides in goiter and other conditions; even the iodine used in preparing patients for operations would increase the iodine content of the thyroid in a short time, so that one might expect such variations in the action of a given thyroid preparation fed to tadpoles as appear in these experiments.

In this connection it is interesting to note (Table II) that Thyroid 20 with 4.31 mg. of iodine was only slightly more active than No. 5 with 1.31 mg. of iodine. Two possibilities have to be considered here. First, No. 20 may have active iodine slightly greater than 1.31 mg. and the balance present as inactive iodine. Second, No. 5 with 1.31 mg. of iodine might represent the maximum possible effect under the conditions of the experiment and a larger quantity of active thyroid iodine could produce no greater effect.

Of course with the lower iodine contents the variations in effects might well come within the limits of errors of observation. Also the percentage error would be greater in the iodine determinations, accidents of feeding, etc.

Our conclusions as to the effect of feeding desiccated thyroid to tadpoles agree in general with those of Lenhart. The action of the thyroid depends not upon a specific stimulus to differentiation but upon a stimulation of metabolism in general in proportion to the active iodine and the quantity consumed. High iodine contents produce

⁴ Marine, D., Demonstration *in Vitro* of the Specific Affinity of Thyroid Cells for Iodin, *Proc. Soc. Exp. Biol. and Med.*, 1915, xii, 132. Marine, D., and Feiss, H. O., The Absorption of Potassium Iodid by Perfused Thyroid Glands and Some of the Factors Modifying It, *J. Pharm. and Exp. Therap.*, 1915, vii, 557.

rapid emaciation, at the same time resulting in differentiation even in tadpoles dying in 8 to 12 days. Low iodine contents result in differentiation at an earlier period than the controls. Tadpoles fed on thyroid with practically no iodine grow better than the controls, in this instance the thyroid acting simply as a food.

Finally, the interest that the results of these experiments may have in connection with the question of function in tumor tissue should be pointed out. To those who hold that tumor lacks the capacity for physiological function, the adenomata of the thyroid could not be consistently regarded as tumors. To those who hold physiological function as a possible property of tumor tissue, the adenomata might be regarded as tumors. Future studies might warrant a recognition of different grades or degrees of tumor. On this basis the fetal adenoma (very little differentiation) might represent a higher degree of tumor than the diffuse colloid or simple adenomatous thyroid in which the adenomatous nodules are present to a great extent throughout the whole gland and are well differentiated. It is certain that there are all grades and degrees of growth and differentiation in the life history of fetal adenomata of the thyroid, from the pure fetal, undifferentiated adenoma with little or no iodine to the simple or colloid adenoma, well differentiated and with varying amounts of iodine approaching that of normal thyroid.

EXPLANATION OF PLATES.

PLATE 38.

FIG. 1. Series A. The condition of the tadpoles at the time of death or after they had been killed in formalin, at the end of 31 days.

PLATE 39.

FIG. 2. Series B. The condition of tadpoles at the time of death or after they had been killed in formalin, at the end of 49 days.

PLATE 40.

FIG. 3. Series C. The effect of desiccated thyroid on *R. catesbiana* tadpoles as compared with the larvæ of *R. pipiens* and *R. clamata*.

FIG. 4. Series D. The effect of desiccated thyroid on tadpoles of different ages.

Series A.



FIG. 1.

(Graham: Adenomata of the Thyroid Gland.)

Series B.



FIG. 2.

Series C.
Control.

14

Samples.



May 25.
12

June 10.
15

June 10.
20



June 10. June 5.

June 5.

June 4.
FIG. 3.

June 4.

Series D.
Control.

Sample.

20



Sample.

Control.

20



FIG. 4.

THE ABSORPTION OF POTASSIUM IODID BY THE THYROID GLAND IN VIVO, FOLLOWING ITS INTRA- VENOUS INJECTION IN CONSTANT AMOUNTS

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In an earlier paper by one of the authors in collaboration with Dr. Feiss,¹ it was shown that artificially perfused and surviving thyroids of dogs take up KI very rapidly and retain it in large amounts; that this activity is not shared by other tissues of the body; that KCN inhibits this activity and that only surviving thyroid cells manifest this phenomenon. At that time two experiments were reported in which 50 mgm. KI were injected intravenously, after having removed a control lobe of the thyroid. The lobes exposed to the KI for one hour showed practically the same affinity for this salt as was found in the in vitro perfusions.

In the present communication we will record the results obtained from a series of 33 experiments in which the KI was introduced intravenously. The plan of these experiments was as follows: In all but four experiments, dogs with grossly enlarged thyroids were used. After ligating the renal vessels of both kidneys and removing one lobe of the thyroid as a control, 50 mgm. KI in and 1 cc. distilled water was injected into the internal jugular vein, or one of its branches, below the thyroid area. Ether for anesthesia was the only drug used and in each case the usual aseptic technique was followed. The animals were allowed to live for periods of 5 minutes, 10 minutes, 1 hour, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours and 30 hours following the injection of KI. In four experiments—two of 5 minutes duration, and two of 10 minutes duration—the renal vessels were not ligated. Then the iodized lobes were removed,

¹ The absorption of potassium iodid by perfused thyroid glands and some of the factors modifying it. *J. Pharmacol. and Exp. Therap.*, 1915, vii, 557.

TABLE 1

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	WEIGHT DOG	WEIGHT OF CONTROL LOBE		IODIN CONTROL PER GRAM	TOTAL I CONTROL	WEIGHT OF IODIZED LOBE		IODIN PER GRAM IODIZED	TOTAL I IODIZED	IODIN INCREASE PER GRAM DRIED	THYROID WEIGHT PER KILOGRAM OF BODY WEIGHT	HISTOLOGY	I PER GRAM DRIED- SPLEEN	I PER GRAM DRIED- LIVER
			Fresh	Dried			Fresh	Dried							
					gram	gram			gram	gram					
a30(A298)	5 minutes	5.00	10.5	2.10	0.00	0.00	13.0	2.6	0.71	1.84	0.71	0.38	Mod-Hyperpl.	trace	trace
32(A325)	5 minutes	9.20	4.0	0.87	2.36	2.05	3.0	0.8	2.61	2.06	0.25	3.06	Colloid	0.062	0.03
33(A326)	5 minutes	5.70	1.0	0.19	1.23	0.23	0.8	0.2	1.63	0.25	0.40	7.12	Normal	0.040	0.03
b30(A323)	10 minutes	6.2	3.5	0.74	0.79	0.58	4.8	0.9	1.38	1.28	0.59	1.30	Early Hyperpl.	0.06	0.02
31(A324)	10 minutes	10.4	3.5	0.61	1.50	0.92	3.3	0.6	2.42	1.35	0.92	3.18	Normal	0.02	0.02
1(A249)	1 hour		25.5	5.10	0.32	1.63	29.0	5.8	0.77	4.47	0.45		Mod-Hyperpl.	0.06	0.03
2(A291)	1 hour		31.0	6.20	0.12	0.74	25.0	5.0	0.48	2.40	0.36		Mkd-Hyperpl.	0.06	0.03
3(A296)	4 hours	11.1	32.0	6.20	trace		69.5	13.9	0.95	13.20	0.95	0.15	Mod-Hyperpl.	0.00	0.00
4(A295)	4 hours	7.0	4.0	0.80	0.86	0.69	4.0	0.8	1.20	0.96	0.34	1.74	Colloid-Goiter	0.00	0.02
27(A320)	4 hours	10.5	29.0	5.74	0.00		25.0	4.4	0.80	3.49	0.80	0.41	Probable cancer areas	0.00	0.00
5(A297)	8 hours	13.0	66.0	13.20	0.18	2.38	49.5	9.9	0.62	6.14	0.44	0.26	Early-Hyperpl.	0.00	0.00
6(A299)	8 hours	10.2	62.5	17.30	0.38	6.57	52.0	12.6	0.57	7.18	0.19	0.19	Early-Hyperpl.	0.00	0.00
25(A318)	8 hours	6.0	28.0	6.92	3.38	23.37	21.0	5.6	4.00	22.47	0.62	0.28	Colloid	0.00	0.00
26(A319)	8 hours	14.5	11.5	2.51	0.15	0.38	12.5	2.7	1.11	3.01	0.96	1.15	Mod-Hyperpl. (un- comp)	0.00	0.00
7(A300)	12 hours	8.8	5.5	1.10	trace		6.5	1.3	0.28	0.36	0.28	1.34	Mkd-Hyperpl.	0.02	0.00
8(A301)	12 hours	6.4	9.5	1.90	trace		5.0	1.0	0.93	0.93	0.93	1.27	Mod-Mkd-Hyperpl.	0.00	0.00
23(A316)	12 hours	4.7	18.0	3.53	0.09	0.32	18.0	3.8	1.23	4.67	1.14	0.26	Mod-Mkd-Hyperpl.	0.00	0.00
24(A317)	12 hours	5.4	66.5	15.09	0.15	2.26	58.0	9.4	0.62	5.85	0.47	0.09	Comp-Colloid Mod.	0.00	0.00
9(A302)	16 hours	12.4	10.0	2.00	1.11	2.22	9.0	1.4	1.54	2.16	0.43	1.37	Colloid	0.00	0.00

10(A303)	16 hours	10.4	10.0	2.00	0.49	0.98	9.5	1.9	0.71	1.35	0.22	1.09	Colloid	0.00	0.00
21(A314)	16 hours	9.2	4.0	0.66	0.15	0.99	4.0	0.7	0.62	0.44	0.47	2.30	Mkd-Hyperpl.	0.00	0.00
22(A315)	16 hours	12.0	31.0	6.85	0.46	3.15	26.5	6.8	1.11	7.55	0.65	0.45	Colloid	0.00	0.00
29(A322)	16 hours	11.8	29.0	5.83	0.18	1.05	23.0	5.1	0.92	4.73	0.74	0.51	Colloid-early	0.00	0.00
11(A304)	20 hours	10.8	6.5	1.80	2.28	4.10	7.0	2.0	2.52	3.07	0.24	1.54	Colloid	0.00	0.00
12(A307)	20 hours	15.0	15.0	3.08	0.12	0.37	14.5	3.4	0.77	2.61	0.65	1.03	Mod-Hyperpl.	0.00	0.00
20(A313)	20 hours	7.2	8.5	1.09	0.62	0.67	9.5	1.4	1.11	1.57	0.49	0.75	Colloid	0.00	0.00
28(A321)	20 hours	28.6	17.5	4.19	0.68	2.85	21.5	5.6	0.68	3.84		1.33	Colloid-early	0.00	0.00
13(A305)	24 hours	6.1	10.5	2.42	0.36	0.87	15.0	3.4	0.74	2.49	0.38	0.40	Colloid	0.00	0.00
14(A306)	24 hours	8.8	13.0	2.35	0.31	0.73	19.5	3.8	1.04	3.93	0.73	0.45	Mod-Hyperpl.	0.00	0.00
17(A310)	24 hours	7.5	10.5	2.38	1.69	4.01	15.5	4.1	2.21	9.06	0.52	0.48	Colloid	0.00	0.00
18(A311)	24 hours	12.9	7.0	1.23	0.00		7.0	1.3	0.46	0.59	0.46	1.84	Mkd-Hyperpl.	0.00	0.00
15(A308)	30 hours	8.6	100.0	17.1	0.00		71.0	12.8	0.62	7.91	0.62	0.12	Mkd-Hyperpl. (com- plie)	0.00	0.00
16(A309)	30 hours	6.6	21.0	3.8	0.02	0.06	15.5	2.9	1.85	5.27	1.70	0.42	Colloid-Mod	0.00	0.00

weighed and small sections taken for histology, and the remainder of the thyroid, together with pieces of liver and spleen desiccated for iodine determinations. As shown in the complete tabulation (table 1) the thyroid lobes used varied markedly in size, in iodine content and physiologic activity, as indicated by the range of histological appearances from quiescent or colloid, to marked active hyperplasia.

As shown in the *in vitro* perfusions the amount of KI absorbed necessarily varies with the surface exposed (size of glands) and the stage of physiological activity (colloid or normal glands showing the least increase in iodine). Any analysis of the quantities of iodine absorbed from a given dose must take into consideration both the size and the stage of physiologic activity of the glands used.

In all experiments there was a great increase in the iodine content of the thyroid lobe thus exposed to KI just as was observed in the *in vitro* perfusions. There are variations in the amounts retained by the thyroid from the constant amount (50 mgm.) offered, and some of the factors which might be related to these variations have been analyzed as follows:

1. Relation of the duration of the perfusion to the amount of KI retained. The principal data bearing on this point have been grouped in the following table, both as to duration of the perfusion and the histologic condition of the glands used—whether active hyperplasias or quiescent glands:

DURATION OF EXPERIMENT	HYPERPLASIAS		COLLOID GLANDS	
	Number of experiments	Average increase in I per gram dried in milligram	Number of experiments	Average increase in I per gram dried in milligram
5 minutes	1	0.71	2	0.32
10 minutes	2	0.75		
1 hour	3	0.53		
4 hours	2	0.87	1	0.34
8 hours	3	0.53	1	0.62
12 hours	4	0.70		
16 hours	2	0.60	3	0.43
20 hours	2	0.57	1	0.24
24 hours	2	0.59	2	0.45
30 hours	2	1.16		

The most striking features brought out in this tabulation are, (1) that the absorption of KI is so rapid during the first few minutes that the slight further increase during the rest of the experiment is masked, and (2), that it varies directly with the degree of active hyperplasia present or inversely with the original iodine content, and therefore is identical in all essentials with the results obtained in the *in vitro* perfusions. Concerning the first point, viz., the rapidity of absorption, it was a surprise to us to find so small a difference between a 5-minute or a 10-minute and a 30-hour perfusion, although the rapidity of the storage of iodine by the gland has been many times and from many different angles emphasized.

This series of experiments indicate that the absorption from the blood is practically instantaneous. In the earlier experiments the kidneys were removed from the circulation partly to bring this series of experiments into relation with the *in vitro* perfusions, and partly to eliminate any loss of KI through the kidneys. This precaution was found to have been unnecessary. In the four experiments where the renal vessels were not ligated no appreciable difference was observed in the percentage of iodine absorbed. The affinity of the thyroid for iodine salts is so great that the loss through the kidney is negligible when iodine is administered in physiological doses for thyroid effects. Even when given in large doses (a decigram of KI) it is doubtful whether the renal factor modifies the amount retained by the thyroid. The thyroid has such an extraordinary affinity for iodine and the other tissues have such a slight affinity for it, that the intravenous injection of iodine salts in the living animal may truly be designated as "*in vivo*" perfusion of the thyroid.

2. In sharp contrast with the thyroid, the liver and spleen show no retention of KI. Samples of liver and spleen were examined for iodine in each experiment, and only in the experiments of 1 hour or less duration was it detected. Traces of iodine were detected in the unwashed tissues of all such experiments just as it was found in the unwashed spleens and kidneys of the *in vitro* perfusions, but even traces could not be detected

when the tissue was thoroughly freed of blood. In all the experiments of 4 hours or longer, no detectable amounts of iodine were found in the liver and spleen.

SUMMARY

There is apparently no difference between *in vitro* and "*in vivo*" perfusions as regards the percentage of iodine absorbed. The absorption is practically instantaneous in each case. Maximum thyroid effects are produced by such exceedingly small amounts of iodine and the gland has such an extraordinary affinity for salts of iodine, that its loss through the kidney may be considered negligible, and this probably holds true for all other body tissues. The size of the gland and the stage of physiological activity modify the amount of KI absorbed apparently to the same degree whether it is introduced by *in vitro* perfusion or injected intravenously in the living animal.

The liver and spleen show no retention of KI, whether introduced by *in vitro* perfusion or by intravenous injection. With constant amounts of KI introduced and with glands of similar degrees of physiologic activity, there is no noteworthy difference in the percentage absorbed, whether the "*in vivo*" perfusion lasts 1 hour or 30 hours. There must be some slight increase in the amount of iodine absorbed from a single dose in the succeeding minutes or hours of a given experiment, but it was not sufficiently marked to be detected as an increase in the iodine content of the thyroid, in this series of glands with the methods employed; although after an hour it was not present in detectable amounts in the circulation.

THE TRANSPLANTATION OF DUCT- LESS GLANDS

WITH REFERENCE TO PERMANENCE
AND FUNCTION *

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The transplantation of normal or tumor tissue is at present attracting wide interest among experimental physiologists and pathologists because of the many fundamental biologic problems which earlier work, both with tumor and normal tissue, uncovered. Among these problems probably the two that are being most actively investigated at present are (1) whether specific nerves (either secretory or regulatory) are necessary for the survival, growth and function of transplanted tissues, and (2) the problem of the reaction of the host to transplanted foreign tissues. For this work the so-called endocrine glands have obvious advantages over glands with external secretions, or the various connective tissues and, indeed, over tumor tissue.

In the course of our work during the past three years, we have studied the transplantation of ovary, suprarenal (cortex and medulla), spleen, parathyroid and thyroid of rabbits. Because the thyroid has several great advantages, namely, its accessibility, its wide range of morphologic changes, which are easily interpreted, and its specific iodine reaction, all useful in checking and controlling results, we have devoted more time and effort to the study of this tissue, and

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the summary to follow is based for the most part on our experiments with the thyroid gland. Our experience has been confined wholly to autotransplantation and homotransplantation, and for the sake of clearness it seems best to present the data under these two divisions rather than according to the tissues used.

AUTOTRANSPLANTATION

We have made nine experiments with ovarian tissue, transplanting in the subcutaneous tissues of the abdomen after removal of both ovaries. In all cases the stroma, interstitial cells and graafian follicles showed survival and growth over periods varying from thirty-four to 219 days. Mature graafian follicles were recovered from two rabbits, associated with active hyperemia of the uterus and the typical phenomena of rut.

A point of practical importance which we have observed in the older transplants is the presence of hemorrhagic cysts, due to the fact that the ripened follicles rupture into themselves instead of onto a free surface as occurs in the normal, and these cysts ultimately produce pressure atrophy of the ovarian tissue. Apart from this complication, our work confirms that of many others that these autotransplants are permanent and show all the evidences of functional activity.

As a part of other experiments, we have made six autotransplantations of spleen tissue in the subcutaneous tissue of the abdomen, all of which were absorbed in twelve days.

Parathyroid tissue has accidentally been transplanted many times with the thyroid, and we have often found active normal looking parathyroid tissue in thyroid transplants when examined microscopically.

In the first 100 rabbits used, thyroid was successfully transplanted into ovary, suprarenal, spleen, jugular vein, muscle, subperitoneal tissues and into the subcutaneous tissues of the neck, chest and abdomen. In the second 100 rabbits, transplantations were made uniformly in the subcutaneous tissue of the abdomen, modified by one or more of the following conditions: with the thyroids intact, partially and completely removed; with and without removal of spleen suprarenals, ovaries, and testes; with and without the administration of phosphorus, and with and without the

administration of iodine using both normal and hyperplastic thyroids. Of these factors, the removal of a large part of the thyroid gland or the administration of iodine materially modifies the growth and activity of the autotransplants.

Autotransplants uniformly "take" and "grow," the amount of growth being determined by the amount of thyroid removed and also by the administration of iodine or desiccated thyroid. Cristiani and von Eiselsberg also observed that removal of the thyroid caused compensatory hyperplasia of transplants irrespective of their location in the body. We can confirm this observation. In every instance, it was found that the histologic condition of the transplant was identical with that of the thyroid gland. Both undergo hyperplasia simultaneously and to the same degree, and both involute simultaneously and to the same degree.

Following the administration of iodine, transplants take up and retain it to the same degree as the thyroid gland. Many authors have stated that transplants of thyroid were permanent. We have observed transplants for more than a year through the phases of spontaneous and induced hyperplasia and involution, and can confirm the statement that they are permanent irrespective of their location.

Inasmuch as such transplanted thyroid tissue undergoes all the morphologic variations associated with growth and function that are observed in nontransplanted thyroid tissue, and inasmuch as transplanted thyroid shows the same reactions with iodine and the same storage of iodine as nontransplanted thyroid, we believe that this is sufficient evidence that such transplants may grow, involute or function equally as well as nontransplanted thyroid. We cannot accept the belief held by some observers that specific nerves, whether secretory or regulatory, are necessary for normal growth or functional activity of the thyroid. The evidence obtained from these observations suggests that the thyroid is truly a blood gland in that the stimuli causing either increased or decreased activity may reach it directly by way of the blood stream. These conclusions are based on the study of 289 autothyroid transplantations in 141 rabbits observed during periods varying from three to 381 days. In forty-one rabbits, only autotransplantations were made, and in 100 both autotransplantations and

homotransplantations were made; in thirty-two of this 100, negative homotransplantations had preceded the positive autotransplantations.

HOMOTRANSPLANTATION

We have made twenty-six homotransplantations of sexually mature ovarian tissue, all but one of which showed complete absorption of the ovarian structure except for the interstitial and luteal cells. The one exception was probably an instance of the failure of the host to react to the foreign tissue in the usual way. The fact that the lipoid cells of the ovary can survive upward of 193 days, while the stroma and egg cells undergo absorption in a few weeks, shows that in the lipoid-containing tissue we are dealing with a different order of cells against which the host reacts very slowly, if at all. There is evidence from the standpoint of transplantation, as well as from that of embryology and morphology, that the cells of the suprarenal cortex belong to this series also. Repeated homotransplantations into the same animal of these lipoid-containing cells of the ovary would probably determine whether or not the host eventually develops a resistance to this tissue also. Up to the present we have not made sufficient experiments to determine this point. The two important facts observed in this series of homotransplantations of the ovary are that: (1) the host reacts in the usual way and usual time to the egg and stroma cells, and (2) the host reacts very feebly to the lipoid containing cells.

We have made eighteen homotransplantations of the spleen, all of which were absorbed in twelve days.

Homotransplantations of thyroid were made in spleen, bonemarrow, suprarenal, ovary, testes, liver, muscle and subcutaneous tissue of neck and abdomen. Purely for the convenience of subsequent examinations, we have used the subcutaneous tissues of the abdomen for all transplants during the past two years.

Up to the present we have made 567 homotransplantations in 205 rabbits of various ages. In 105 of these only homografts were made. As in the case of thyroid autografts, the conditions have been varied as follows: with and without removal of the thyroid, ovaries, spleen and testes; with and without administration of iodine and phosphorus, using phosphorized and iodized thyroids separately and together.

A tabulation of the results shows that complete absorption may take place as early as the tenth day, and usually occurs before the thirtieth day in cases which have not been previously homotransplanted. As is well known, repeated homotransplantation markedly accelerates the destruction of homografts, due to the development of an immunity, the nature of which is little understood. On the other hand, we have under observation two rabbits containing homotransplants of more than a year's duration, which grossly and microscopically resemble autografts. Between these two extremes, there are all gradations in the rate of destruction. This to our minds is the most significant fact we have observed, and, in reviewing the literature, we have been unable to find reports dealing specifically with these variations in large series of nontumor transplantations.

A thorough understanding of these variations would go far toward explaining the causes of the failure of homotransplantation and, as the students of tumor point out, it is also the most important problem confronting them. It seems well established that these variations depend on the development of an immunity to a foreign protein (tissue), and tumor investigators have shown that the degree of foreignness of the tissue used is the most important factor in its development. A study of our thyroid material suggests that these variations in the rate of absorption may be due to intrinsic differences in the reaction of the host, quite apart from and in addition to the other important factor of the foreignness of the tissue used. In the series of 205 rabbits about 92 per cent. destroyed initial homografts in from ten to thirty days, and subsequent homografts, as is well known, were destroyed more rapidly. The remaining 8 per cent. of the rabbits showed strikingly less rapid reactions even though the same gland was used for the two groups in most instances, and the factor of blood relationship could be considered most remote. The following instance, of which there are several others in the series, may be mentioned in some detail in support of the foregoing statement.

The thyroid slightly hyperplastic, of Rabbit 233 was transplanted into three rabbits (Rabbits 226, 227 and 229), May 8, 1915. Within thirty-seven days the transplant in Rabbit 226 was completely absorbed, and within ninety days the trans-

plant in Rabbit 227 was absorbed, while the transplant in Rabbit 229 is still large and active after 400 days. June 14, 1915, the thyroid of Rabbit 263 was also transplanted into Rabbits 226, 227 and 229 and, in addition, as initial grafts into six other rabbits, and as second homografts into four additional rabbits. This thyroid (from Rabbit 263) had disappeared from all but one of the thirteen rabbits at the time of the first examination, the single exception being Rabbit 229, which still has a homotransplant of 400 days from Rabbit 223. This second positive homograft was removed at the two hundred and forty-third day, and in both gross and microscopic appearances had all the characteristics of an autograft.

These experiments show that one has to deal with variations in the resistance of animals, which is quite independent of the thyroid used. It is clear, therefore, that when one finds an animal in which an initial homograft is positive, subsequent homografts from unrelated animals may remain and act as autografts. On the other hand, we have never seen a positive homograft following an initial negative homograft, no matter what the age, sex or blood relationship of the rabbits used.

Turning now to the second factor in the variations of the rate of absorption, namely, the degree of foreignness of the tissue used, there is evidence that one can modify the rate of absorption by modifying the condition of the host and also the chemistry and physiologic activity of the thyroid used, as is demonstrated in the following experiments:

In three rabbits with thyroids intact (Rabbits 237, 238 and 239), potassium iodid was given in 20 mg. doses for two weeks previous to transplantation. These three were then partially thyroidectomized and transplanted on the left side from the thyroid of Rabbit 254, in which marked hyperplasia had been induced by a previous partial thyroidectomy. The same hyperplastic thyroid was transplanted into the left side of two other rabbits (Rabbits 255 and 256) which had had similar previous partial thyroidectomies and whose thyroid stumps were hyperplastic. The iodized and quiescent thyroid of Rabbit 237 was at the same time transplanted into the right side of all five (Rabbits 237, 238, 239, 255 and 256). Subsequent examination of the thyroid grafts made from the hyperplastic thyroid into both the iodized and noniodized rabbits shows that in the two rabbits (255 and 256) with previous partial thyroidectomies absorption occurred in thirty days; while in the three iodized rabbits (237, 238 and 239) the grafts disappeared in one (Rabbit 237) after fifty

days; in the second (Rabbit 239) it was positive and was recovered at necropsy 144 days later and in the third (Rabbit 238), the graft was positive at 149 days but had disappeared at the two hundred and first day.

Subsequent examination of the right thyroid grafts made with the iodized thyroid of Rabbit 237 shows that in the non-iodized rabbits (255 and 256) absorption occurred in the usual time, while in the two iodized rabbits (238 and 239), this thyroid was removed in one (Rabbit 239) at necropsy 144 days later, and in Rabbit 238 the transplant is still large and active at 382 days and resembles in all respects an autograft.

This series of experiments shows clearly that when iodized thyroid is homografted into iodized rabbits, the rate of destruction is markedly decreased. As iodine is a physiologic constituent of thyroid, and as these experiments show that its previous administration to both donor and host delays the rapidity of absorption of homografts, it seems certain that it is possible to modify the usual reaction of the host by strictly physiologic means. While iodine favorably affects the thyroid, there is no evidence that it has a similar action on other homografted tissues. Its influence on the fate of the thyroid homograft suggests, however, that it may be possible to modify the host's reaction to other homografted tissues through one or more of their specific chemical constituents.

SUMMARY

Concerning autografts we have been able to confirm the conclusions of others that thyroid when transplanted shows all the evidence of growth, function and permanence, and to the same degree, as does the non-transplanted thyroid. This work also shows that specific nerves, whether secretory or regulatory, are not necessary either for the control of growth or of function in the case of the thyroid.

Concerning the behavior of thyroid homografts, it seems established that both the host and the tissue used for the grafts modify their duration. These two factors may be quite independent, antagonistic to or helpful to each other. In the case of the thyroid this reaction may be modified by iodine.

Lastly, the future of tissue transplantation as a therapeutic means rests on a solution of the problem

of the homograft, and it is also certain that whatever headway is made in overcoming the obstacles to homografting will to an equal degree be applicable to the solution of the tumor problem.

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THE THYROID GLAND IN RELATION TO GYNECOLOGY AND OBSTETRICS

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THE relation of the thyroid to the sex organs in the female is the most ancient and classical illustration of the interrelation of the function of glands with internal secretions. Known to the ancients in its crudest external manifestation, a subject of their daily gossip, it has passed down through the ages. Even today, in spite of the records of thousands of observations and experiments, we must confess to a very meager insight into the fundamental physiological processes involved.

Experimental approach is difficult. The lower animals lack the obvious manifestations seen in man. The search for hormones, chemical activators and depressors is still in its infancy. However, many facts of importance are known and I shall review some of them on the following pages.

Of the several possible tissues whose activities may be concerned, the thyroid, adrenal, and sex glands appear most important. The adrenal medulla is important because of the effect of its hormone on all sympathetic nerve endings; the adrenal cortex because embryologically it is identical with the interstitial cells of the ovary. Both the adrenal cortex and interstitial cells are characterized by a high lipin content, the physiological rôle of which is unknown, although there are many facts to indicate that it is of great importance. Both tissues reciprocate in their physiological hyperplasias, and reasoning from analogy with the male, these lipin-rich interstitial cells probably play an important rôle in the development and maintenance of the secondary sexual characteristics.

Ancestrally the thyroid exists in the chordates in two forms — as an elaborate ventral midline pharyngeal glandular groove, the so-called “endostyle” in all the lower chordates — tunicates, amphioxus and ammocetes (larval lampreys), and as the familiar ductless thyroid in all higher chordates — adult lampreys, fish, amphibians, reptiles, birds, and mammals. Fortunately, the animal (lam-

prey) in which the transition from endostyle to thyroid can be followed, still exists, otherwise this extraordinary metamorphosis could not have been established.

The thyroid then is primarily a pharyngeal gland, probably closely related both to digestion and respiration and in the thyroid of higher animals, all its known activities are still intimately related to metabolism. Variations in size within physiological limits are characteristic of all three of these tissues. In man, the dog and the cat it is the thyroid which shows the largest variations, while in rabbits the adrenal cortex and ovaries show greater variations in size than the thyroid.

It is usually stated that the thyroids in women are larger per unit of body weight than in men. This is in general true, so far as anatomical statistics can go, but it has misled some authors to imply that the difference is inherent, while in truth it is acquired and can be entirely controlled.

All the known physiological activity of the thyroid is associated with iodine. Used in ignorance from the most remote times in the form of sponge ash, sea-weed, crude salt, etc., in the treatment of thyroid enlargement, discovered as an element in 1811 by the Frenchman Courtois, first knowingly used in medicine by the Geneva physician, Coindet, in 1820, it remained for Baumann, of Freiburg, to discover it as a normal constituent of the thyroid in 1895. Iodine is usually present only in traces in the thyroid at birth, unless the mother has been given iodine, when it is enormously increased. In the normal gland there are wide variations in the iodine content. The average is about 0.2 per cent of the dried weight or from 10 to 15 milligrams in the whole gland. The iodine-containing hormone is bound with the globulin of the colloid from which Kendall has recently been able to separate it by alkaline hydrolysis and to obtain it in crystalline form. Its chemical nature is unknown, though Kendall thinks it is a di-iodo-indol.

In general, the iodine of the thyroid varies with the amount of colloid. Iodine is markedly decreased in the developmental stages of all goiters and following the administration of its soluble salts it is almost instantly taken up by the thyroid. Physiologically this iodine-containing hormone is the most powerful activator of metabolism known. This effect appears to be brought about through stimulation of the oxidation processes and if the work of Asher and Flack and of Cannon and his co-workers is confirmed, the influence of epinephrin is very important in augmenting its action and vice versa the influence of the thyroid hormone greatly augments the pressor activity of epinephrin. With our present knowledge we attempt to explain the instances of increased functional activity of the gland on the basis of an increased demand for thyroid activity or what amounts to the same thing, an increased demand for the iodine-containing hormone.

Thyroid enlargements appear to be compensatory or work hypertrophies and are readily controlled or prevented by the administration of very minute amounts of iodine. Removal of the thyroid is followed by similar basic symptoms in both young and adult animals. They all depend upon depression of the various activities of tissues and a decrease in total metabolism. In the young, this change manifests itself in arrested growth and development, sexual, somatic and mental—the so-called cretin. In the adult, loss of sexual functions, increased fatty deposits, mental deterioration, anæmia, and malnutrition of all the tissues are the most prominent manifestations. There is no evidence of selective action or that certain organs or groups of organs are more affected than others. Superficially this might seem to be the case because certain symptoms, like those of the nervous system or genital system, are more obvious and earlier recognized.

Removal of the thyroid like removal of the ovaries or adrenals is usually accompanied by persistence of the thymus, spleen enlargement, enlargement of the lymph glands and a lymphocytosis. Nothing is known as to the cause of these changes. Removal of a large portion of the adrenals in rabbits causes

slight, though definite, hypertrophy of the thyroid and lymphoid hyperplasia. This is also seen in Addison's disease in man, and might be explained as part of the adrenal-thyroid interrelation.

Removal of the adrenals also causes hypertrophy of the interstitial tissue of the ovaries in rabbits, and removal of the ovaries causes hypertrophy of the adrenal cortex or even of subcutaneous transplants of adrenal cortex.

Removal of the ovaries in animals probably tends to decrease the activity of the thyroid. There is no evidence that this is a direct effect. The various attempts to establish a direct relationship between the thyroid and ovaries by a comparison of the influence of extracts on metabolism have given negative or doubtful results. Through the study of cryptorchids, and experiments of ligating the vas deferens it has been definitely established that the interstitial lipin-rich cells of the testes largely determine the male secondary sexual characters. In the case of the ovary it is not possible to separate the oogenic cells from the interstitial cells, but the attempts thus far made suggest that these cells play a very important and similar rôle in the secondary sex characters of the female.

Nevertheless, it is an outstanding fact that in man thyroid hyperplasia is many times (6 to 8) more common in the female during and after adolescence than in the male during and after adolescence. Up to this period sex makes no difference in the incidence. Congenital goiter is not influenced by sex and in all the lower animals sex likewise has no influence, the incidence remaining the same at all periods of life.

In the human subject, the periods when thyroid enlargements most frequently occur are at puberty, during menstruation and during pregnancy. During each of these periods the body metabolism is increased and as it is a major function of the thyroid to stimulate oxidation processes in the body, it is probable that the heightened metabolism is of thyroid origin and the enlargement of the thyroid at these times is a true work hypertrophy. This view is supported by the facts that supplying the iodine-containing

hormone artificially or even iodine, from which the gland can elaborate its own hormone in increased amounts, prevents the hypertrophy, and in any developing hypertrophy of the gland the iodine is decreased. In rut and pregnancy of the lower animals these changes are too slight for certain detection, though many authors have reported mild degrees of thyroid hypertrophy in both rut and pregnancy. I have given considerable attention to the study of this feature and have never been able to detect any change in size, histological appearance, or iodine content greater than the range of changes found normally in either sex unassociated with sexual activity. An increase in metabolism occurs in animals also during rut and pregnancy and, therefore, some increase in thyroid activity is probable, but it is too slight to be recognized by morphological or chemical changes in the thyroid as can often be done in man.

The degree of change in the thyroid during puberty, menstruation, and pregnancy is normally slight, amounting to no more than the enlargement incident to the increased blood supply. Occasionally hypertrophy of the epithelium occurs, and always there is some decrease in the iodine content. Cellular hypertrophy is not possible until a great drop in the iodine has taken place. In the dog, ox, sheep, pig, and man it has to fall to less than 0.1 per cent as comparing with a normal of over 0.2 per cent of the dried weight. These anatomical changes are identical with those which occur in developing goiters, and in goiter districts it is at these periods that simple goiter most frequently develops. The development of great enlargements of the thyroid at these periods merely means the coincidence of the cyclic sexual factor with the continuously operating causal agent of simple goiter and must not be confused with the slight increase in activity or better, the slight temporary insufficiency of the thyroid of sexual origin.

It is possible that the same chemical disturbance initiates the thyroid change, both in sexual activity and in simple goiter, the difference being one of degree. This is purely a speculation, for experimental work so far

has furnished no suggestive lead as to the exciting cause of either. Nor has the study of menstrual disturbances, of the pathological physiology of pregnancy or of diseases of the genital tract thrown any light on the nature of the thyroid reaction associated with sexual activity.

The extensive study of the relation of the sex glands to Basedow's disease likewise has given no clue to the nature of the thyroid sex gland interrelation; though the incidence as regards sex is similar to that of simple goiter.

To summarize, it may be stated that there is evidence in man of a thyroid sex gland interrelation recognizable in the female in association with the development of secondary sexual characters, with menstruation and with pregnancy and also in the male at puberty, but to a very slight degree. The meager evidence available would tend to indicate that the interstitial cells of the ovary and perhaps, also, the adrenal cortex play a major rôle in this relation in the female, as certainly the cells of Leydig do in the male.

The thyroid enlargement is of the nature of a work hypertrophy to stimulate metabolism identical in appearance and so far as we know, different only in degree from that seen in simple goiter. Both of these reactions can be controlled and prevented either indirectly by giving iodine or directly by giving the iodine-containing hormone in physiological doses.

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TRANSPLANTATION OF THE THYMUS IN RABBITS—RELATION OF THE THYMUS TO SEXUAL MATURITY

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THE objects of these experiments were to obtain more definite data on the transplantability of the thymus; and if transplantable in accessible locations, to utilize this means of studying its behavior in relation to sexual maturity and breeding.

The thymus normally undergoes a striking atrophy or involution at puberty. It seems well established by the work of Paton and Goodall, Henderson, Calzolari and others, that removal of the gonads before puberty delays thymus involution; that thymus removal hastens sexual maturity in rabbits; and that animals allowed to breed show earlier thymus involution than those not so used.

In our experiments, we have removed all the main thymus mass (exposing the gland by splitting the sternum to the third rib) except the upper portion of one of the cervical cornua, and have transplanted small (2 to 3 mm.) fragments into the subcutaneous tissue of the abdomen. Contrary to the results of Renton that transplants in the subcutaneous tissues did not survive, we have found that it can be readily transplanted in this location in sexually immature rabbits. Whether the peritoneal or subperitoneal tissues are still more favorable, as some authors state, we have no data.

As with the spleen, only autotransplants have survived. Immediately after thymectomy, as above described, two autotransplants were placed in the subcutaneous tissue of the abdomen of each of 8 rabbits. Each rabbit was between three and one-half and four months old. Six of these rabbits have been observed for three months, and the transplants examined directly at monthly intervals. Two females were kept with one of the males. Examination at the end of the first month showed that both were pregnant, which is earlier than rabbits usually breed. At the gross examination, the transplants in all three seemed negative, though the enlarging breasts made the examinations unsatisfactory. One transplant area was removed from each and examined histologically. In one female, there was an active transplant, while in the other female and the male the transplants had undergone nearly complete absorption. Of the remaining five, two died before the end of the first month. The remaining three, (two females and one male) had active transplants, the male and one female having large 4 mm. transplants, showing clearly that growth had occurred.

At the beginning of the second month, the female with the large thymus transplants was bred, and at the end of the second month these transplants could not be found, though on account of the lactating breasts the examination was not satisfactory. The male with large transplants at the end of the first month had active transplants, possibly larger than at the first examination. The unbred female also had active transplants.

Again at the examination after three months, the male and female (now over seven months old) kept isolated, still had active transplants. One from each was removed for histologic examination. Microscopically, these are encapsulated vascular masses of compact lymphoid tissue. There is no increase in fibrous tissue about thymus transplants as is usually seen around spleen grafts.

The remaining four (used for breeding) were also examined at the end of three months, and the five remaining transplant areas removed. Definite but small masses of thymus lymphoid cells were found in two.

These experiments, which are preliminary, show that in sexually immature rabbits, fragments of thymus autotransplanted into the subcutaneous tissue of the abdomen after thymectomy may "take," grow, and survive. There is clear though scant evidence in confirmation of other observers' results, that thymus removal hastens sexual maturity. Also, as others have found, utilization of rabbits for breeding hastens involution of the thymus. Our experiments show that this applies to the transplanted thymus as well, and this suggests that a specific nerve influence is not essential for these involutionary changes.

STUDY OF A CASE OF DIABETES INSIPIDUS WITH SPECIAL REFERENCE TO THE MECHANISM OF THE DIURESIS AND OF THE ACTION OF PITUITARY EXTRACT ON IT *

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CLEVELAND

The case of diabetes insipidus reported in this paper presented, on account of the high degree of the diuresis, an unusually good opportunity for the study of certain points in connection with the mechanism of the diuresis and the influence on it of extracts of the posterior lobe of the pituitary body. Although it was not possible to induce the patient to remain long enough in the hospital to enable us to complete our program, the results obtained seem worthy of being recorded.

The manner in which the excretion of water by the kidney is regulated has formed the subject of recent papers by Priestley¹ and by Haldane and Priestley.² They find, as T. M. Wilson,³ working under the direction of one of us, previously showed, that the drinking of water is followed by a small diminution of the specific conductivity of the blood serum. According to Wilson, the meaning of this would seem to be that "when the relative volume of serum is increased (e. g., by drinking water) the serum becomes more dilute as regards salts, and therefore has a diminished specific conductivity. When the serum diminishes in amount, water seems to pass out of it in greater proportion than salts." Haldane and Priestley were unable to demonstrate any change in the relative volume of the plasma which could be detected by estimating the percentage hemoglobin content.⁴ But Wilson, using a more delicate test, the determination of the relative volume of corpuscles and plasma by the electrical method,⁵ was able to show that coincident with the decrease in the conductivity of the serum there was a slight increase in its volume as compared with that of the corpuscles.

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1. Priestley: *Jour. Physiol.*, 1916, **50**, 304.

2. Haldane and Priestley: *Jour. Physiol.*, 1916, **50**, 296.

3. Wilson, T. M.: *Am. Jour. Physiol.*, 1905, **13**, 150.

4. Hemoglobin estimations and blood counts carried on throughout the period of observation on our patient failed to show any definite correspondence with the intake of water or the diuresis.

5. Stewart, G. N.: *Jour. Physiol.*, 1899, **24**, 356.

It seemed to us not unlikely that during the great and abrupt changes in the diuresis produced in our case by posterior lobe extract, or by withholding water, or allowing it in the enormous amounts habitually taken by the patient, a greater and therefore more easily detectable effect might be produced on the blood than was possible in any normal individual.

An attempt was made by measuring the blood flow through the hands to determine whether posterior lobe extract produced any effect on the superficial vessels which might afford support to the view that it affects the diuresis by a change of caliber of the renal vessels.

The ability of the kidney to excrete a concentrated urine, and its behavior to so-called "functional tests," were also investigated.

An attempt to review the theories which have at various times been held in regard to the mechanism of diabetes insipidus would be out of place. It will suffice for our purpose to refer to a few of the investigations which have seemed to connect the condition with a change in the activity of the pituitary body.

The frequent association of diabetes insipidus with tumors and injuries about the base of the brain, and the demonstration by Claude Bernard that puncture of the floor of the fourth ventricle occasionally produced a polyuria without glycosuria, led many observers to the belief that the disease was in some way dependent on the central nervous system. Magnus and Schäfer⁶ demonstrated that extracts of the hypophysis caused an increased urine output. Schäfer and Herring⁷ showed the diuretic effect of hypophyseal extract to be a property of the pars intermedia. They claimed the diuretic action was due to a direct stimulation of the kidney cells, associated with local dilatation of the kidney vessels. The work of Schäfer and Herring has been pretty generally accepted. These observations suggested that diabetes insipidus might be a manifestation of a hyperactive pituitary body.

Cushing⁸ later pointed out that patients and animals frequently developed a diuresis following the removal of the posterior lobe of the pituitary body which was not unlike diabetes insipidus. Frank⁹ observed a patient in whom diabetes insipidus had developed coincidentally with the lodgment in the sella turcica of a bullet which encroached on the posterior lobe of the pituitary body. Lewis and Mathews¹⁰ produced an analogous condition in dogs by inserting foreign material in the sella which encroached on the pituitary body. Such observations cast doubt on the conception that diabetes insipidus was the result of an overfunctioning posterior lobe. Cushing,⁸ von den Velden and Farmi¹¹ expressed the view that diabetes insipidus was the result of an under-functioning pituitary body.

There are in the literature a number of instances of patients suffering from diabetes insipidus who have experienced amelioration in the symptoms after intramuscular injection of posterior lobe extract. Recently recorded instances

6. Magnus and Schäfer: Jour. Physiol., 1901-1902, **27**, 9 (Proc. Physiol. Soc.).

7. Schäfer and Herring: Phil. Tr. Roy. Soc., 1906, **199**, B. p. 1.

8. Cushing: The Pituitary Body and Its Disorders, 1912.

9. Frank: Berl. klin. Wchnschr., 1912, **49**, 393.

10. Lewis and Mathews: THE ARCHIVES INT. MED., 1915, **15**, 451.

11. Von den Velden and Farmi: Berl. klin. Wchnschr., 1913, **50**, 2083.

are those of Motzfeldt¹² and Eisner.¹³ Motzfeldt reported three cases in which the urine output was cut down markedly, and in one of the patients there had been almost complete relief from the symptoms by the administration of the fresh posterior portion of the gland by mouth.

REPORT OF CASE

History.—C. F., woman, single, aged 31, was admitted to the Lakeside Hospital medical service, Nov. 13, 1916.

The patient's health had always been good until the present trouble began. There was no history of acquired or congenital syphilis. She was well and working regularly four years prior to admission, when she noticed rather suddenly that she was passing larger quantities of urine than usual and that her thirst was unquenchable. She was forced to give up her work within a short time. She gradually became very weak, but did not lose weight. Since that time her condition has remained much the same. Some days the urine output and the thirst have been greater than on other days. The urine output, as near as we could determine from the history, has ranged from 8 to 16 liters daily. During this time she has had several attacks in which she would be confined to bed with shortness of breath and swollen legs. She has also suffered intensely with a "breaking out" on the hands and arms, worse in winter, when the pain and itching are almost intolerable.

Physical Examination.—If it were not for an anemia the patient would appear in robust health. She is well developed and slightly inclined toward adiposity. The skin was extremely dry and showed a high grade of anemia. On the dorsum of the forearms and hands there was a papulosquamous eruption. The skin in these areas was extremely rough and dry, with multiple fissures. There was some slight oozing in spots, with scab formation (dermatitis hiemalis). The examination of the eyes, pupils and eyegrounds was entirely negative. There was a venous hum; no thyroid enlargement. The examination of the chest was negative, aside from rather extreme cardiac enlargement. No murmurs or adventitious sounds were heard over the precordium. The pulse was regular and there was no elevation of blood pressure. There was a moderate increase in the volume of the liver, but it was not tender. Examination of the nervous system revealed no abnormal findings.

The urine was pale, of large quantity, and low specific gravity. There was a faint trace of albumin in the admission specimen, but subsequent examinations revealed none. There were no casts.

The blood showed: hemoglobin (Sahli), 35 per cent.; white blood cells, 7,800; red blood cells, 3,700,000.

Lumbar puncture revealed no increase in pressure; fluid normal in appearance; two cells per cubic centimeter. The Noguchi and Wassermann tests were negative.

Roentgenograms of the region about the sella turcica showed no increase in the size.

One sugar determination was made on the blood of this patient by the method of Lewis and Benedict. It was found to be 0.168 gm. per hundred c.c. This would represent a distinct hyperglycemia, but the patient had never shown sugar in the urine, so immediately following the above determination she was given 250 gm. of dextrose by mouth and subsequent urine specimens were examined for sugar. They were all negative. The reduction in the blood was probably not due to sugar.

12. Motzfeldt: Boston Med. and Surg. Jour., 1916, **174**, 644.

13. Eisner: Deutsch. Arch. f. klin. Med., 1916, **120**, 438.

METHODS OF STUDY

Considerable difficulty was encountered in the management of this case. The patient frequently drank 2 liters of water at a time and voided a similar amount of urine. Strict supervision was required. She was placed in a room by herself and was constantly watched by a nurse. Four nurses were engaged in her care. Water was measured into flasks in the laboratory and delivered to the room as they were needed. Other fluids were measured and figured in with the intake. Food was given to the patient at 8 a. m., 12 m., and 5 p. m., no food being allowed between meals. The diet was general except on days indicated in Figure 1 on which water was restricted. On these days the regular Mosenthal renal test diet was given. The days when the renal test diet was being taken, 1,000 c.c. of water was given with each meal instead of the stipulated amount. No water was allowed between meals and no particular attempt was made to limit the water after 8 p. m. on these days. The weight was obtained at 8 a. m. without permitting the patient to drink or to void after 7 a. m.

Blood specimens were always taken from the median cephalic vein into a small amount of oxalate, for blood ureas, sugar, etc. For hematocrit and electrical conductivity measurements, it was taken into a flask with beads and immediately defibrinated.

EFFECT OF POSTERIOR LOBE EXTRACT

The profound effect of intramuscular injection of a commercial extract of a pituitary solution of the posterior lobe of the pituitary body on the water intake and the urine output is clearly brought out in Figure 1. As nearly as could be determined, the effect was evident in about one hour following the administration. For the first three days no posterior lobe extract was administered. On the first day it will be noticed there was considerable disparity between the intake and output. This was undoubtedly due to an error in counting the flasks on the part of attendants, who were not accustomed to the routine, as the weight remained constant. On the following day the water intake was restricted for twenty-four hours, but the patient excreted about 5,400 c.c. more urine in this day than she took fluids. Figure 2 shows that on this second day the patient lost $11\frac{1}{2}$ pounds in weight. This loss is accounted for by the excess urine output over the water intake. On the following day, December 5, the patient kept some of the ingested fluid and recovered some of her admission weight. On the three following days, December 6 to 8, the patient was given posterior lobe extract, 1 c.c., three times daily, intramuscularly. Although she was allowed water at discretion, the tremendous drop in water intake and urine output will be noted. Her thirst was greatly diminished and the weight remained practically constant for the three days. December 8, or the third day of the posterior lobe extract period the fluids were again restricted to a much greater degree than on December 4, but on the 4th she passed a great excess of urine over the intake and was very uncomfortable, while on the 8th under posterior lobe extract the output was only slightly in excess of the intake, the

weight fell about 3 pounds and the patient suffered only moderate discomfort from thirst. From December 9 to 11, inclusive, no posterior lobe extract was given and no fluid restriction imposed. The output and intake reached a higher figure than we have ever seen recorded. On each of the following three days, or from the 12th to the 14th, inclusive, the patient was given 1 c.c. of posterior lobe extract, and it will be noticed that the intake and the output were about one-third of what they were when she received 3 c.c. daily. The weight gradually crept up on these three days to near her admission weight. December 15 the fluids were again restricted and the patient given 3 c.c. of posterior lobe extract. From 5 p. m. until the following morning, December 16, at 10 a. m., restriction of water was made as severe as

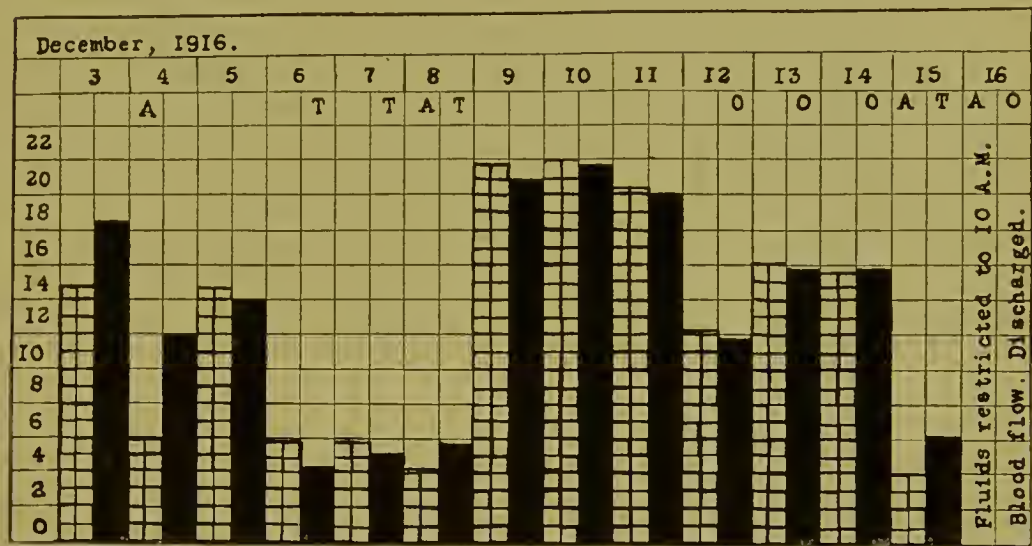


Fig. 1.—Quantity of intake and output per day expressed in liters. Hatched column, intake; solid column, output. On days marked with an A, fluids were restricted; other days there was no fluid restriction. Days indicated by a T, posterior lobe extract was given in 1 c.c. dose, intramuscularly, three times daily. Days marked with an O, a single dose of posterior lobe extract was given intramuscularly.

possible to see whether any effect could be produced on the conductivity of the serum and its relative volume. The patient in the twenty-six hours ending at 10 a. m., December 16, lost 6 pounds in weight.

While the antidiuretic effect of posterior lobe extract given intramuscularly was extremely evident, we were not able to show that the drug prepared for oral administration exerted the same beneficial results, although the results have not been completely negative. Our experience with the oral administration of fresh posterior lobes from cattle has been so far about the same as with the oral administration of the prepared extract. Of course, it is obviously impossible to continue the injections, for it would require at least three a day to keep

the patient in comfort, as the effect lasts only from five to seven hours. After that time there is complete escape from the effect. Larger doses, 2 c.c. per injection, were no more satisfactory; in fact, less so, for the effect did not last any longer and there were more unpleasant accessory actions, as increased irritability of the bladder, with frequent desire to urinate.

As regards the effect of the drug on the blood pressure, the systolic pressure was never found above 130 mm. of mercury, and the diastolic ranged near 75 mm. Although this patient had a hypertrophied heart with dilatation, we felt there was no contraindication to continuing the injections. No change was noted in the blood pressure following the administration.

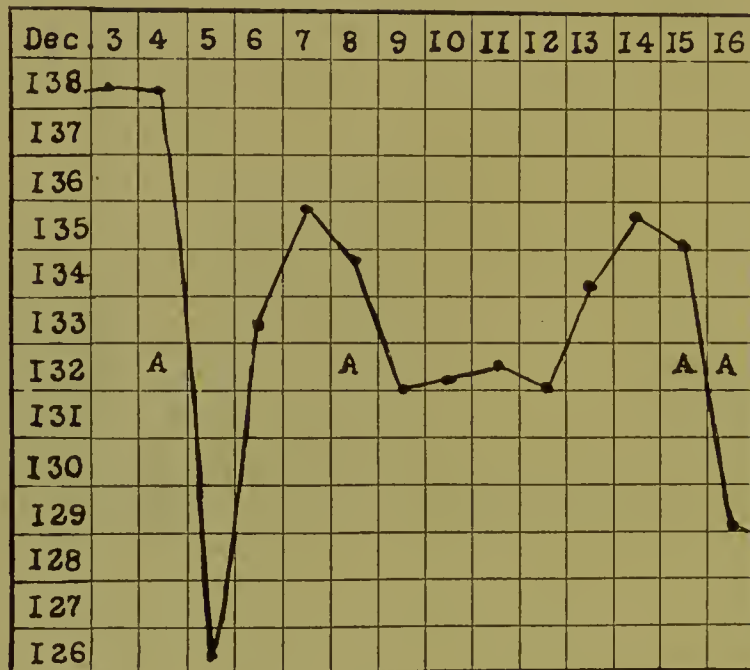


Fig. 2.—Represents the weight of the patient expressed in pounds. These weights were obtained at 8 a. m. On days marked with an A, fluids were restricted.

CONDUCTIVITY AND RELATIVE VOLUME OF SERUM

Table 1 shows the results of observations on three specimens of blood and serum. Although the number of specimens was not as great as could have been wished, owing to the reluctance of the patient, the results seem to show clearly enough a definite although slight increase in the conductivity of the serum and a correspondingly slight decrease in its volume relatively to that of the corpuscles when the intake of water was greatly diminished, either by actual restriction or by giving posterior lobe extract, which lessened the thirst. December 11, as will be seen from Figure 1, the patient took in and excreted over 20 liters

of water. December 15, under posterior lobe extract and slight water restriction (Table 6), the intake was only 4 liters and the excretion by the kidney $6\frac{1}{2}$ liters.

In the seventeen hours preceding the collection of the blood specimen, December 16, water was restricted as much as possible (little more than 1 liter). It will be seen (Table 1) that the conductivity of the serum of December 16 is the highest, and its relative volume the lowest of the three specimens, while the corresponding numbers for December 16. The relative volume of the serum was determined both by the electrical method and the hematocrit. While the hematocrit only gives relative results on account of the difficulty of reaching an absolutely constant end-point, the fact that the longer the centrifugalization is continued the nearer do its readings come to those of the electrical method, confirms the accuracy, at least for comparative purposes, of the results.

TABLE 1.—SHOWING CONDUCTIVITY AND RELATIVE VOLUME OF BLOOD SERUM

Date	K $\times 10^4$ at 5 C.		Percentage Volume of Serum by	
	Blood	Serum	Electrical Method	Hematocrit*
12/11	54.6	78.6	82.9	79 ($6\frac{1}{2}$ min.) 80 ($13\frac{1}{2}$ min.)
12/15	52.4	79.5	80.2	76.5 (7 min.) 77.5 (12 min.) 78 (17 min.)
12/16	53.7	82.5	79.0	73 (14 min.) 74.8 (21 min.) 75.7 (21 min.) 76.5 (36 min.)

* Turned at rate of 4,000 revolutions per minute.

The differences in conductivity do not seem to be even as great as those observed by Wilson and by Haldane and Priestley, in whose observations the variations in the quantity of water transported by the blood were much smaller. It must be remembered that the blood is simply the transportation system for the water, and the amount in transit at any moment is no more an index of the amount transported per hour or day than the number of cars of wheat on a railway on a given day are an index to the size of the wheat crop. While it would not be justifiable from so small a number of observations to draw the conclusion that the kidney is stimulated to increase its excretion of water by an even smaller excess of water in the blood in diabetes insipidus than in health, the suggestion may be made that if the kidney is really abnormally sensitive to water excess, so that the threshold of the stimulus is lowered, it would afford an explanation of the condition. It must be remembered, however, that in this patient the pro-

portion of plasma in the blood is greater than normal, so that, on the assumption that the total volume of blood is not less than normal, the addition of a given amount of water to the blood would not dilute the plasma so much or increase its relative volume so much as in a normal person.

MEASUREMENT OF THE BLOOD FLOW IN THE HANDS

This was done on four days by the method previously described by one of us.¹⁴ The idea was to see whether posterior lobe extract caused any definite effect on the blood flow through the superficial vessels at the time when it was causing a decrease in the diuresis. If its anti-diuretic effect was associated with an alteration in the flow through the superficial vessels (not associated with a change in the heart's action, which there was no reason to suspect), it was argued that this would render it more probable that vascular reactions were occurring elsewhere, as in the kidney, which might account for the diminution in

TABLE 2.—BLOOD FLOW IN THE HANDS—

Date	Pulse Rate	Temperature (C.) of				Volume of Hands in C.c.		Heat Given Off in Grams, Calories		
		Room	Arterial Blood	Calorimeters		Right	Left	Right	Left	In Min
				Right	Left					
12/11/16	78	24.0	36.92	31.81	31.75	347	360	573	761	10
		24.0		31.88	31.86			725	1,032	10
12/14/16	75	24.4	36.58	31.75	31.72	357	362	237	270	10
		25.4						348	315	10
12/15/16	84	24.0	36.90	32.03	32.07	366	358	933	1,108	10
		24.5		32.17	32.26			930	1,092	10
12/16/16	78	24.2	36.90	31.30	31.35	357	358	602	754	10
		24.8		31.35	31.45			652	855	10

the excretion of the urine. A dilatation in peripheral areas might very well be associated with a renal constriction. Unfortunately, the existing dermatitis on the patient's hands rendered them peculiarly susceptible to contact with water, so that the uniformity of results in the control observations was less than is usually seen under hospital conditions. She said the water increased the irritation, and she had a similar objection to protecting the skin by oil or vaselin, so that it proved impossible to get as many observations as we desired. The skin affection and the anemia, which is always associated with subnormal hand flow, rendered the exposed parts abnormally susceptible to changes of temperature in the wards. In spite of these drawbacks, however, the four experiments carried out, the condensed results of which are given in Table 2, do seem to indicate an increase in the hand flow under the influence of posterior pituitary lobe extract.

14. Stewart, G. N.: Heart, 1911, **3**, 33.

The highest flows were seen on December 15, when the antidiuretic effect was well established. Not only was the flow decidedly better than in the two control experiments (on December 11 and December 14) when no posterior lobe extract was being given, but the flow was steady practically from the time the hands were put into the calorimeter, and did not slowly creep up over a considerable period of time, as in the control experiments. This slow, almost reluctant, increase is a feature of the flow in the hand when its vessels have an abnormally great tendency to vasoconstriction, and when the maximum flow is reached it will then, in any case, be small. Both criteria, therefore, indicate that on December 15 the posterior lobe extract had to a considerable extent overcome the tendency to cutaneous vasoconstriction. The measurement of December 16, although it gave a somewhat lower flow than the first control measurement (of December 11), is not really out of harmony with this. The patient had received no posterior lobe extract

A PATIENT WITH DIABETES INSIPIDUS

Blood Flow in Gm. per Minute		Flow per 100 C.c. of Hand per Minute		Period in Calorimeter	Remarks
Right	Left	Right	Left		
2.46 5.98	16.35 22.66	3.59 4.60	4.54 6.29	First ten minutes Second ten minutes	No posterior lobe extract
5.45 7.94	6.17 7.14	1.52 2.22	1.70 1.97	Second ten minutes Third ten minutes	No posterior lobe extract
1.29 1.84	25.49 26.15	5.81 5.96	7.12 7.30	First ten minutes Second ten minutes	Posterior lobe extract action
1.94 3.05	15.09 17.43	3.34 3.65	4.21 4.86	Second ten minutes Third ten minutes	Doubtful posterior lobe extract action

for seventeen hours, but one hour before the measurement was started she was given 1 c.c. Also she had lost six pounds in weight during the previous period of water restriction, and much of the water she was now taking was not being excreted by the kidneys, but was going back into the tissues. Furthermore, she had been sitting around the ward, as she wanted to leave the hospital, and said that she had been cold all the morning. Her hands were cold when they were put into the bath. The patient said she had noticed that after receiving the injections the skin of the hands, which was habitually dry, became more moist and that her hands, which were habitually cold, became warmer. We confirmed the statement that sweat appeared distinctly on the hands when she was under the influence of posterior lobe extract, while at other times they were extremely dry.

The dermatitis was worse on the left hand and arm, which probably accounts for the greater flow in that hand on December 11, 15, and 16.

That the inequality was due to a vasomotor and not to a mechanical difference is shown clearly in the experiment of December 14, when, under the influence of markedly increased vasoconstriction due to cold (two of the fingers on the right hand had been "dead" earlier in the day, she said), the inequality disappeared. If the flow for the second and third ten-minute periods of the experiment be added, they are practically equal for the two hands.

ABILITY OF THE KIDNEY TO CONCENTRATE THE URINE

Erich Meyer¹⁵ advanced the hypothesis that the diuresis of diabetes insipidus is primarily the result of a disease of the kidney. Such a view has been maintained essentially on the assumption that the kidneys of patients afflicted with diabetes insipidus were not able to elevate the concentration of the urine. Mosenthal¹⁶ has recently offered evidence in favor of Meyer's hypothesis. Histologic examination of the kidneys in diabetes insipidus lends no support to the view that the condition is due to any structural alterations in these organs.

There are now quite a few instances recorded in the literature of patients afflicted with diabetes insipidus whose kidneys have shown definite concentrating ability. Fitz,¹⁷ in this country, has reported a case in which he was able to demonstrate a moderate ability on the part of the kidney to elevate the specific gravity of the urine. Both Motzfeld¹² and Eisner,¹³ by the use of pituitary extract injections, were able to show the same thing, only to a more marked degree.

Our patient had a severe anemia, which in itself is sufficient to impair the ability of the kidneys to concentrate the urine, as has been recently shown by Mosenthal,¹⁶ Christian¹⁸ and unpublished data accumulated by one of us (C). Probably if it had not been for this disturbing element our results would have been more striking than they are.

In studying the concentrating ability of the kidneys the patient was put on a standard Mosenthal renal test diet. This diet contains 13.4 gm. of nitrogen, 8.5 gm. of sodium chlorid and 1,760 c.c. of fluids. No alterations were made in the procedure except to elevate the quantity of water served at each meal. Days when these test diets were run have been indicated in Figure 1 by the term "water restriction." On all of the days when the patient was taking the renal test diet no attempt was made to restrict the patient's water intake after

15. Meyer, Erich: *Deutsch. Arch. f. klin. Med.*, 1905, **83**, 1; *Ztschr. f. klin. Med.*, 1912, **74**, 352.

16. Mosenthal: *THE ARCHIVES INT. MED.*, 1915, **16**, 733.

17. Fitz: *THE ARCHIVES INT. MED.*, 1914, **14**, 706.

18. Christian: *THE ARCHIVES INT. MED.*, 1916, **18**, 429.

8 p. m. So in reality we only made twelve-hour observations; but for completeness the "night" urine was balanced for estimating total nitrogen and chlorid output.

Table 3 shows the response of the patient's kidneys to a renal test meal on November 27, six days before our more accurately controlled period began. No posterior lobe extract was given. The diuresis was not so great on this day as it had been on other days, and the specific gravity of the night urine was distinctly higher than usual. The excretion of nitrogen and salt was perfectly normal. Although there was moderate fixation of the specific gravity, the percentage excretion of salt and nitrogen would indicate definite concentrating ability on the part of the kidney.

TABLE 3.—RESPONSE OF PATIENT'S KIDNEYS TO A RENAL TEST MEAL WITHOUT POSTERIOR LOBE EXTRACT

Time	Amount, O.c.	Sp. Gr	Chlorids	Per Cent.	Nitrogen	Per Cent.
8 to 10	1,226	1.000				
10 to 12	955	1.000				
12 to 2	1,150	1.004				
2 to 4	750	1.003				
4 to 6	400	1.005				
6 to 8	615	1.003				
Total day.....	5,096	4.59	0.09	5.79	0.11
Night, 8 to 8.....	1,315	1.007	3.29	0.24	5.67	0.43
Total output.....	6,411	7.88	11.46	
Intake.....	6,810	8.5	13.4	
Balance.....	+399	+0.62	+1.94	

It was thought that if extreme water restriction were imposed with the patient under the régime of a renal test meal and no posterior lobe extract, that the concentrating ability of the kidney might be made more evident than in Table 3. Table 4 shows the result of such an observation on December 4. This was the second day of the observation period and during the first ten hours the patient was restricted to 1,760 c.c. of water. It was necessary to terminate the observation at 6 p. m., and allow water. It will be seen that the patient excreted 5,400 c.c. more fluid than she took in and lost 11½ pounds in weight, as shown in Figure 2, on December 5. The nitrogen and salt were not determined in this instance, but it will be noticed that the concentrating ability of the kidney was only slightly in evidence.

TABLE 4.—URINE CONCENTRATION UNDER EXTREME WATER RESTRICTION WITHOUT POSTERIOR LOBE EXTRACT

Time	Amount, C.c.	Specific Gravity
8 to 10.....	1,885	1.003
10 to 12.....	722	1.002
12 to 2.....	1,270	1.005
2 to 4.....	1,075	1.007
4 to 6.....	910	1.007
Total day.....	6,962	
Night, 6 to 8.....	5,600	1.002
Total output.....	11,562	
Intake.....	6,160	
Balance.....	-5,402	

TABLE 5.—SHOWING RESPONSE OF PATIENT'S KIDNEYS TO A RENAL TEST MEAL

Time	Amount, C.c.	Sp. Gr.	Chlorids	Per Cent.	Nitrogen	Per Cent.
8 to 10.....	320	1.001*				
10 to 12.....	100	1.011				
12 to 2.....	100	1.013				
2 to 4.....	155	1.010				
4 to 6.....	135	1.015				
6 to 8.....	207	1.006				
Total day.....	1,017	2.01	0.2	3.27	0.3
Night, 8 to 8.....	4,370	3.49	0.08	5.87	0.13
Total output.....	5,387	5.50	9.04	
Intake.....	4,210	4.40	12.60	
Balance.....	-1,177	-1.10	+3.54	

* 1 c.c. posterior lobe extract 9 a. m., 3 p. m. and 9 p. m.

Table 5 represents the response of the patient's kidneys to a renal test meal given December 8 of our period of observation. The patient, as shown in the table, received three intramuscular injections of posterior lobe extract of 1 c.c. each, at 9 a. m., 3 p. m. and 9 p. m. In this experiment, as well as in the subsequent one, it will be noticed that

the 9 a. m. dose of posterior lobe extract did not show any marked effect until after 10 a. m. It will be seen, however, that there is a distinct ability on the part of this patient's kidneys to elevate the specific gravity of the urine. The nitrogen and salt elimination appear normal. There seems to be a tendency, however, for the solids to be excreted in greater quantities during the night. But this was just as apparent when the patient was not under the influence of the drug. The effect of the dose given at 9 p. m. usually wore off between 2 and 4 a. m.

Table 6 shows the results of a test diet day on December 15 of our period of observation. The results confirm those shown in Table 5, although the patient did not seem to get so much under the effect of the three doses of posterior lobe extract as she did in that instance.

TABLE 6.—RESULTS ON URINE OF A TEST DIET

Time	Amount, C.c.	Sp. Gr.	Chlorids	Per Cent.	Nitrogen	Per Cent.
8 to 10	1,010	1.001*				
10 to 12	230	1.012				
12 to 2	922	1.004				
2 to 4	360	1.007				
4 to 6	162	1.012				
6 to 8	124	1.007				
Total day.....	2,808	4.40	0.15	3.75	0.13
Night, 8 to 8.....	3,710	1.001	4.04	0.11	5.35	0.14
Total output.....	6,518	8.44	9.10	
Intake.....	4,010	4.25	11.88	
Balance.....	-2,508	-4.19	+2.78	

* 1 c.c. posterior lobe extract 9 a. m., 3 p. m. and 9 p. m.

There was a phenolsulphonephthalein excretion of 70 per cent. in two hours. Blood urea was twice estimated. Two hours after an essentially carbohydrate breakfast it was 0.017 gm. per 100 c.c. The urea index at the same time was 157. Another blood urea estimation two hours following a protein meal gave 0.028 gm. per 100 c.c. and a urea index of 81.

We can conclude, then, that with posterior lobe injections this patient's kidneys had ample ability to concentrate the urine. Without the drug there was evidence that the kidneys had only a meager ability to elevate the specific gravity. Other functional tests all indicated a normal kidney excretion.

SUMMARY

The regulation of the excretion of water by the kidney was studied in a case of diabetes insipidus. It was supposed that on account of the high degree of the diuresis, the great quantity of water ingested and transported, and the marked diminution in the excretion and ingestion caused by pituitary posterior lobe extract, the conditions for such a study would be unusually favorable.

The conductivity of the blood serum was slightly increased and the relative volume of serum slightly diminished when the water excretion was lessened by posterior lobe extract or by water restriction.

The blood flow in the hands seemed to be increased during the antidiuretic action of posterior lobe extract. This, so far as it goes, supports the view that a vascular effect in the opposite direction on the renal vessels may be responsible for the diminution in the urine excretion.

It was shown that under the action of posterior lobe extract the kidneys had the power of effecting a considerable concentration of the urine. Other kidney functional tests gave a normal response. Accordingly, no indication was obtained that the condition was in any way associated with a pathologic alteration in the kidney.

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FIVE HUNDRED AND THIRTY-FIVE NORTH DEARBORN STREET
CHICAGO

The Effect of Bandaging of the Legs
on the Rate of Blood Flow
in the Feet

G. N. STEWART, M.D.
CLEVELAND

THE EFFECT OF BANDAGING OF THE LEGS ON THE RATE OF BLOOD FLOW IN THE FEET *

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CLEVELAND

It has been suggested by some observers of the condition known as "trench-foot," which was so common among the soldiers in the trenches in Belgium and France, especially during the first winter of the war, that obstruction of the venous return by the puttees worn by the British troops was an important contributory factor.

The observations which form the subject of this paper are concerned only with the effect of relatively short applications of the puttees. Having been made in a laboratory on a normal man, they do not reproduce what is probably an important condition for the development of trench-foot, if the pressure of the puttees has anything to do with the condition, namely, the swelling under an already tight, wet, and dirty bandage. So far as the technic of the observations is concerned, it would have been easy enough to study the circulatory changes at first hand on soldiers. Not having been able to do this (and of course in such matters the military authorities must be the final judges as to what military exigencies will allow), the writer is, for the present at least, obliged to content himself with publishing a few specimen results on a normal man, which he had hoped to compare with data obtained by clinical studies of the actual condition at different stages. Since, however, so far as I am aware, no investigation of the influence of such bandages applied to the legs on the flow of blood in the feet has been published, and since such measurements have an interest in other relations, the application of bandages to limbs being so common in surgery and in certain medical procedures, it seems worth while to record the result of this preliminary study.

The observations were made on M. C., a healthy man, aged 26 years, weight 165 pounds, height 5 feet, 10 inches. Numerous measurements of the blood flow in his hands and feet have been made in the past few years, so that the range of variation of the flow under the conditions of the observations is well known.¹ In all the experiments

* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University School of Medicine, Cleveland.

1. Stewart, G. N.: Jour. Exper. Med., **18**, 354, 372.

the amount of water in the calorimeters was 2,550 c.c. The puttees were always applied over the trousers, which were tucked in in the usual way.

October 22. M. C., four hours after breakfast, put his feet in the bath at 11:02 and in calorimeters at 11:18 a. m. The pulse was 90. At 11:40 a puttee was put on the right leg with the ordinary degree of firmness. At

TABLE 1

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
11:17	31.08	31.22		12:05	31.795	31.59	24.1
11:20	31.19	31.24	24.3	12:07	31.82	31.63	
11:22	31.24	31.27	24.3	12:09	31.85	31.65	24.1
11:24	31.28	31.29	24.3	12:11	31.88	31.675	
11:26	31.32	31.30		12:13	31.91	31.69	24.1
11:28	31.36	31.34	24.4	12:15	31.96	31.74	
11:30	31.395	31.36		12:17	32.00	31.775	24.1
11:32	31.41	31.37	24.2	12:19	32.06	31.83	
11:36	31.44	31.39	24.2	12:21	32.10	31.86	24.0
11:38	31.47	31.40	24.3	12:23	32.14	31.90	
11:40	31.495	31.43		12:25	32.19	31.94	24.1
11:43	31.52	31.44	24.2	12:27	32.23	31.97	
11:45	31.53	31.45		12:29	32.295	32.04	24.1
11:47	31.55	31.455	24.2	12:31	32.34	32.08	
11:49	31.58	31.46	24.2	12:33	32.39	32.125	24.1
11:51	31.60	31.465		12:36	32.43	32.16	
11:53	31.62	31.47	24.2	12:38	32.47	32.18	24.1
11:55	31.63	31.475		12:40	32.49	32.195	
11:57	31.66	31.485	24.2	12:42	32.50	32.22	24.1
11:59	31.68	31.50		12:44	32.52	32.24	
12:01	31.72	31.54	24.2	12:46	32.44	32.19	
12:03*	31.76	31.57		1:01	32.12	31.87	

* Right foot feels more comfortable than left, and a little warmer.

12:23 the puttee was rapidly taken off. At 12:44 the feet were removed from the calorimeters.

The cooling of the calorimeters in fifteen minutes was 0.32 C. The volume of the right foot was 1,171 c.c., of the left 1,128 c.c. The water equivalent of the calorimeters with their contents was, right 3,616 c.c., left 3,584 c.c. The rectal temperature was 36.89 C.

In Table 1 is given an experiment in which a puttee was applied only to the right leg. Since the pressure is not the only effect which

the bandage can exert on the blood flow, but the warmth may also be important, in other experiments puttees were applied to both legs, but with different degrees of tightness.

In this experiment the flow in the feet was less than in the others cited, possibly because the subject had been going about for some time with bare feet and legs, completing the preparations for the observations; probably also in part because the experiment was made several hours after a meal. The taking of food, since it increases metabolism, must also increase the heat loss, and therefore the vasodilatation of the skin. The initial vasoconstriction due to exposure of the feet

TABLE 2

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
2:37	30.89	30.95		3:10	32.95	32.875	
2:40	30.99	31.03	24.0	3:12	33.01	32.95	24.0
2:42	31.20	31.20	24.1	3:14	33.09	33.03	24.1
2:44	31.40	31.39	24.2	3:16	33.18	33.13	
2:46	31.57	31.55	24.3	3:18	33.26	33.19	24.1
2:48	31.73	31.71	24.3	3:20	33.36	33.30	
2:50	31.89	31.86		3:22	33.45	33.37	24.1
2:52	32.02	32.99		3:24	33.53	33.46	
2:56	32.24	32.18	24.1	3:26	33.61	33.55	24.1
2:58	32.33	32.28	24.1	3:28	33.70	33.65	
3:00	32.45	32.38		3:30	33.78	33.72	24.15
3:02	32.58	32.51	24.0	3:32	33.86	33.785	
3:04	32.72	32.65		3:34	33.81	33.74	
3:06	32.80	32.74	24.0	3:46	33.50	33.43	
3:08	32.88	32.80	24.0				

tended to pass off as the experiment proceeded, the rate of flow increasing in the successive periods. But there was no indication that the puttee on the right leg in any way interfered with the flow in the right foot. The ratio of the minute flow in the left foot to that in the right was almost precisely the same in the eighteen minutes before the application of the puttee as in the forty minutes during which it remained on. The data are displayed in succinct form in Table 5.

In the experiment shown in Table 2 a puttee was applied firmly to the right leg. The degree of pressure of the puttee was about what the soldier commonly employs. On the left leg a similar puttee was applied quite loosely, so that the heat-conserving effect would be the

same as on the right leg, while the pressure was negligible. For the ten minutes before the application of the puttees the flow per minute per 100 c.c. of part was 7.18 gm. for the right and 7.2 gm. for the left foot, practical equality. For twenty minutes with the puttees on, the flow was 6.43 gm. and 6.58 gm. per minute per 100 c.c. For fourteen minutes after removal of the puttees the flow was 8.09 gm. and 8.13 gm. per 100 c.c. per minute for the right and left foot respectively.

TABLE 3

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
2:36	30.87	30.88		3:17	33.33	33.22	24.2
2:38	30.93	30.97	24.1	3:19	33.40	33.27	
2:40	31.09	31.09	24.1	3:21	33.47	33.34	24.2
2:42	31.28	31.27		3:23	33.52	33.42	
2:44	31.42	31.43	24.1	3:25	33.595	33.47	24.2*
2:46	31.58	31.55		3:27	33.65	33.54	24.25
2:48	31.70	31.68	24.0	3:29	33.72	33.59	
2:50	31.85	31.82		3:31	33.78	33.66	24.2
2:52	31.99	31.92	24.1	3:33	33.83	33.72	
2:54	32.12	32.055		3:35	33.90	33.79	24.2
2:56	32.25	32.16	24.1	3:37	33.97	33.86	
3:00	32.41	32.34	24.3	3:39	34.02	33.915	24.2
3:02	32.53	32.45	24.25	3:41	34.07	33.96	
3:05	32.70	32.60	24.3	3:43	34.13	34.02	24.2
3:07	32.805	32.69		3:45	34.19	34.07	
3:09	32.90	32.80	24.25	3:47	34.26	34.14	24.2
3:11	33.00	32.91		3:49	34.30	34.18	
3:13	33.11	33.03	24.2	3:51	34.26	34.12	
3:15	33.22	33.13		4:05	33.90	33.76	

* Left leg felt cool after the puttee was taken off it; the right leg felt warm.

October 26. M. C., had lunch one hour before, and had walked outside for some time. The pulse was 94. The feet were put in the bath at 2:22, and in calorimeters at 2:39 p. m. At 2:52 a puttee was put firmly on the right leg and one slackly on the left leg. At 3:16 the puttees were taken off quickly. At 3:32 the feet were taken out of the calorimeters.

The cooling of the calorimeters in twelve minutes was 0.31 C. The volume of the right foot was 1,180 c.c., of the left 1,128 c.c. The water equivalent of the calorimeters with their contents was, right 3,623 c.c., left 3,385 c.c. The rectal temperature was 37.05 C.

If the twenty minute period is analyzed, it is seen that for the first half of it the flow was 6.84 gm. and 6.97 gm. for the two feet,

respectively; and for the second half 6.13 gm. and 6.3 gm. The diminution in the right foot is not due to pressure of the bandage, since it is the same as the diminution in the left. Removal of the puttees was followed by some increase in flow in both feet, but without disturbance of the relation of equality in the two feet present from the beginning of the experiment. The increased flow cannot be interpreted as due to the vasomotor paralysis following the removal of a tight bandage described by Bier. For the puttee on the right leg was not so tight as to occasion the slightest discomfort, and the increased flow was present in the left foot as well as in the right.

TABLE 4

Time	Temperature of Calorimeters		Room Temperature	Time	Temperature of Calorimeters		Room Temperature
	Right	Left			Right	Left	
11:26	30.92	30.78		12:05	32.46	31.99	24.15
11:29	31.00	30.86	25.2	12:07*	32.56	32.08	24.2
11:31	31.07	30.92	25.2	12:09	32.61	32.14	24.2
11:33	31.17	30.98	25.2	12:11	32.68	32.18	
11:35	31.27	31.07	25.1	12:13	32.73	32.23	24.2
11:37	31.37	31.16	24.7	12:15	32.80	32.28	24.25
11:39	31.49	31.26	24.4	12:17	32.88	32.34	
11:41	31.59	31.34		12:19	32.96	32.40	24.2
11:45	31.71	31.43	24.5	12:21	33.00	32.455	
11:47	31.76	31.45	24.4	12:23	33.07	32.51	24.2
11:49	31.81	31.48	24.3	12:25	33.09	32.55	
11:51	31.895	31.55		12:27	33.13	32.59	24.1
11:53	31.96	31.59	24.1	12:29	33.17	32.64	24.1
11:55	32.04	31.65	24.0	12:31	33.19	32.66	
11:57	32.16	31.755	24.0	12:33	33.20	32.68	24.0
11:59	32.22	31.81	24.0	12:35	33.20	32.68	
12:01	32.28	31.85	24.05	12:37	33.12	32.615	
12:03	32.37	31.91	24.1	12:49	32.81	32.31	

* The right leg was comfortable, the left somewhat tired and uncomfortable.

In the experiment shown in Table 3 the procedure was the same as in the last experiment, except that the puttee on the left leg (put on quite slack) was removed some time before the other, in order to see whether the increased loss of heat from the left leg caused any effect on the flow in the right foot. There was, as a matter of fact, a slight diminution in the flow in the left foot, which might be attributed to vasoconstriction due to increased cooling. If this was the cause,

the action extended reflexly to the right limb, the puttee on which had not been disturbed, as the ratio of the flows in the two feet remained unchanged. There was no indication that the pressure of the puttee on the right leg diminished the flow in the right foot.

October 29. M. C., one hour after lunch, put his feet in the bath at 2:20, in the calorimeters at 2:37 p. m. The pulse was 88. At 2:56 a puttee was

TABLE 5

Date	Pulse Rate	Temperature (C.) of				Volume of Foot in C.c.		Heat Given Off in Gm.—Calories		
		Room	Arterial Blood	Calorimeters		Right	Left	Right	Left	No. Min.
				Right	Left					
10/22	90	24.3	36.29	31.37	31.35	1,171	1,128	2,115	1,792	18
		24.1	21.83	31.67	5,170	4,551	40
		24.1	32.36	32.09	2,680	2,530	19
10/26	94	24.2	36.45	31.61	31.60	1,180	1,128	3,695	3,549	10
		24.05	32.52	32.46	2,855	2,825	10
		24.05	32.99	32.94	2,253	2,248	10
		24.1	33.56	33.49	3,478	3,423	14
10/29	88	24.1	36.36	31.92	31.86	1,190	1,133	3,158	2,913	10
		24.25	32.87	32.78	4,720	4,520	17
		24.2	33.56	33.43	2,051	2,027	10
		24.2	34.04	33.92	3,557	3,516	18
11/ 2	80	24.8	36.34	31.38	31.16	1,171	1,142	2,133	1,905	8
		24.3	31.88	31.54	2,007	1,600	10
		24.1	32.36	31.92	3,743	3,290	16
		24.2	32.90	32.37	2,155	1,923	10
		24.1	33.14	32.60	1,610	1,725	12
11/30	100	20.5	36.41	31.19	31.05	1,171	1,142	6,183	4,602	20
		20.6	31.49	31.23	3,073	2,394	10
		20.9	32.19	31.74	2,759	2,459	10
		21.1	32.60	32.10	1,797	1,675	6
		21.2	33.13	32.57	3,952	3,703	18
		21.0	33.53	32.92	1,121	1,064	6

put firmly on the right leg and one slackly on the left leg. At 3:17 the puttee was rapidly taken off the left leg; at 3:29 off the right leg. The feet were taken out of the calorimeters at 3:49.

The cooling of the calorimeters in fourteen minutes was 0.36 C. The volume of the right foot was 1,190 c.c., of the left 1,133 c.c. The water equivalent of the calorimeters with their contents was, right 3,630 c., left 3,588 c.c. The rectal temperature was 36.96 C.

Indeed, with both puttees on, the flow was slightly and equally increased in both feet, probably on account of the heating effect.

In the next experiment to be quoted (Table 4) the puttee was purposely put on the left leg so tightly as to produce a certain amount of discomfort, while it was applied to the right leg with the ordinary, comfortable degree of pressure. The subject was hungry (four and

TABLE 5—(Continued)

Blood Flow in Gm. per Min.		Flow per 100 O.c. of Foot per Min.		Ratio of Flow in 2 Feet	Remarks
Right	Left	Right	Left		
3.53	22.39	2.26	1.98	1:1.14	Before puttee was put on
2.20	27.37	2.75	2.42	1:1.14	Puttee on right leg
9.83	35.23	3.40	3.12	1:1.09	Puttee off
4.82	81.31	7.18	7.20	1:1.00	Before puttees put on; after eating
7.72	78.67	6.84	6.97	1:1.02	Put. R. firm, L. slack (first 10 minutes)
6.35	71.16	6.13	6.30	1:1.03	Put. R. firm, L. slack (next 10 minutes)
5.51	91.78	8.09	8.13	1:1.00	Puttees off
6.03	71.92	6.64	6.34	1:1.05	Before puttees put on; after eating
6.39	82.52	7.42	7.28	1:1.02	Put. R. firm, L. slack
6.39	76.87	6.83	6.78	1:1.01	Puttee off L. leg
6.64	88.94	7.95	7.85	1:1.01	Puttees off both legs
6.73	51.08	5.10	4.47	1:1.14	Before puttees; after eating
6.00	37.03	4.27	3.24	1:1.31	Put. R. firm, L. too tight (first 10 minutes)
6.31	51.69	5.57	4.52	1:1.23	Put. R. firm, L. too tight (next 16 minutes)
6.60	53.82	5.94	4.71	1:1.26	First 10 minutes after puttees removed
6.58	42.70	3.97	3.73	1:1.06	Next 12 minutes after puttees removed
6.80	47.69	5.62	4.17	1:1.34	Before puttees put on
6.39	51.35	5.92	4.49	1:1.31	Last 10 minutes before puttees
6.64	58.51	6.20	5.12	1:1.21	First 10 minutes with puttees
6.34	71.97	7.46	6.30	1:1.18	Next 6 minutes with puttees
6.37	59.52	6.35	5.21	1:1.21	After tightening tapes on R. leg
6.06	42.34	4.61	3.70	1:1.24	After puttees off

one-half hours after breakfast) and the flow in the feet was less than in the experiments shown in Tables 2 and 3. The flow in the two feet was 5.10 gm. and 4.47 gm., respectively, per 100 c.c. per minute for an eight minute period before the application of the bandages (ratio of left to right 1 to 1.14). For a period of twenty-six minutes with the puttees on the flow was 5.13 gm. and 4.04 gm., respectively

(ratio 1 to 1.27). The cutting down of the flow by the improperly applied puttee on the left leg is evident, although the bandage was not put on so tight as to cause an extreme degree of discomfort.

November 2. M. C., four hours after breakfast, put his feet in the bath at 11:07, in the calorimeters at 11:27:30 a. m. At 11:41 puttees were put firmly on both legs, but the left was much tighter and less comfortable than the right. At 12:11 the puttees were rapidly taken off. The feet were taken out of the calorimeters at 12:35.

The cooling of the calorimeters in twelve minutes was, right 0.31 C., left 0.305 C. The volume of the right foot was 1,171 c.c., of the left 1,142 c.c. The water equivalent of the calorimeters with their contents was, right 3,616 c.c., left 3,595 c.c. The pulse was 80. Rectal temperature was 36.96 C.

If the period is analyzed, the deficit in the left foot is seen to be greatest in the first ten minutes (flow in right foot 4.27 gm. per 100 c.c. per minute, in left 3.24 gm., giving a ratio of 1 to 1.31). The flow in the right foot is also diminished somewhat. In the remaining sixteen minutes of the period of application of the puttees the flow increases in both feet, but relatively more in the left, as the venous pressure rises and forces the block (5.57 gm. for right and 4.52 gm. for left foot, ratio 1 to 1.23). Even with the tight bandage on the left leg, the initial flow in the left foot is soon reestablished. This, however, is in a healthy man, whose cutaneous flow is uniformly good. It seems clear enough that in men who habitually suffer from cold feet, and whose foot flow is therefore normally small, the injudicious application of a puttee might easily cause a harmful reduction in the flow which would render the foot more readily susceptible to the effects of cold and wet, of slight injuries and of the passive congestion associated with standing for long periods in one position. On the other hand, so far as can be judged from experiments of such relatively short duration, a properly adjusted puttee not only causes, under normal conditions, no permanent diminution in the foot flow, but may even somewhat increase the flow, probably largely because of its heat conserving property.

In another experiment (that of November 30) different methods of fastening the puttees were investigated. That on the right leg was tied by tapes an inch wide, that on the left leg was fastened smoothly by a safety pin. The tapes caused a somewhat tighter feeling, according to the subject, than the pin, but both legs felt comfortable. After the first sixteen minutes the tapes were made considerably tighter on the right leg, but not uncomfortably so. As will be seen in Table 5, the tightening of the tapes did not produce any diminution in the flow in the right foot as compared with that in the left, although the flow in both was now diminishing, and diminished still more after removal of the puttees. This last decrease in the flow, seen also in the experiment of November 2, may be due to a vasoconstriction associated with

cooling of the limbs after removal of the bandages. However, a terminal vasoconstriction due to the exposure is not uncommonly seen in long experiments on the foot flow when no bandages have been applied.

SUMMARY

It is shown that a puttee applied with the usual degree of pressure on the leg causes no diminution in the blood flow through the foot. When it is put on so tight that some discomfort is produced the flow in the foot is at first reduced.

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THE LIBERATION OF EPINEPHRIN FROM THE ADRENAL GLANDS BY STIMULATION OF THE SPLANCHNIC NERVES AND BY MASSAGE

STUDIED BY MEANS OF THE DENERVATED EYE REACTION

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The observations of a number of investigators have indicated that during electrical stimulation of the splanchnic nerves, epinephrin passes into the circulation by the adrenal veins. These observations may be divided into two groups: (1) Those in which blood has been collected from the adrenal veins of one animal, and tested for epinephrin by its action on the blood pressure when injected into the veins of another animal (Dreyer (1), Tscheboksaroff (2), or by its action on such isolated tissues as intestine or uterus segments (Stewart (3)). (2) Observations in which the liberation of epinephrin has been deduced from changes in the blood pressure or other reactions in one and the same animal. Thus Joseph and Meltzer (4), found that when one superior cervical ganglion was removed from rabbits, and several days later the peripheral end of the splanchnic nerve stimulated electrically, there resulted in the great majority of cases a variable but unmistakable dilatation of the pupil on the ganglion-free side, while the pupil on the normal side remained practically unchanged. They interpreted this as due to the secretion of the adrenals being thrown into the circulation in sufficient amount to produce a dilatation of the pupil on the ganglion-free side, since it is well known that the pupil on that side is much more sensitive than normal, to the action of adrenalin. (Meltzer and Auer (5)). While by the first group of methods, results

to clamp the inferior vena cava. In a few observations both cava and aorta were clamped. Another simple method is to stop the heart by stimulating the vagus. The eye reaction is never obtained under these conditions, that is to say the pupil of the denervated eye does not dilate in the absence of dilatation of the pupil of the normal eye, nor is the nictitating membrane retracted.

EXPERIMENTS IN WHICH THE VENOUS PATH FROM THE ADRENALS WAS OCCLUDED

A certain amount of dilatation may take place simultaneously in both pupils during occlusion of the inferior vena cava, but this is clearly associated with the accompanying asphyxia, and is easily distinguished from the genuine reaction which involves only the denervated eye. When the clamp is removed from the adrenal vein or the cava, the reaction is obtained in the denervated eye and after precisely the same time interval, allowing for the slight variations in successive observations, as is seen when the splanchnics are stimulated without occlusion of the vessels. It is impossible to interpret this result in any other way than that the epinephrin given off in response to the splanchnic stimulation is lying in the adrenal capillaries or veins ready to move out into the circulation at the moment of removal of the clamp.

Thus in an experiment on a cat, the condensed protocol of which is given in table 6 stimulation of the right splanchnic in the thorax was followed by dilatation of the left (denervated) pupil in 9 seconds, and withdrawal of the nictitating membrane. The inferior cava was then clamped above the diaphragm and kept clamped while the splanchnic was again stimulated. No change occurred in the left eye till 9 seconds after removal of the clamp, when dilatation of the pupil began, followed by retraction of the nictitating. The cava was again clamped and the splanchnic stimulated. Dilatation of the pupil occurred 10 seconds after removal of the clamp. Another stimulation of the splanchnic (with the cava free) gave dilatation of the pupil in 8 seconds from the beginning of stimulation. As the cava had been twice clamped for 30 seconds within an interval of 4 minutes, it is pos-

sible that interference with the circulation in the iris, owing to the fall of blood pressure, might have caused the slight lengthening in the time interval (from 9 to 10 seconds) of the reaction obtained after removal of the clamp. In any case, absolute uniformity in the signalling of the moment at which such a reaction is observed to begin cannot be expected, and the closeness of the agreement of successive readings is really remarkable. Later on in this experiment, although numerous injections of methylene blue and of adrenalin solution had been made in the interval, the reaction caused by stimulation of the splanchnic was still obtained 10 seconds after removing the clamp from the cava. At the very end of the experiment, however, when the animal was obviously in bad condition, the pupil reaction did not occur till 19 seconds from the beginning of stimulation of the splanchnic (with vena cava free). In another experiment on a cat, from which the left superior cervical ganglion had been removed 6 days previously, the time interval between the beginning of stimulation of the splanchnic (in the thorax) and the beginning of dilatation of the pupil (followed by retraction of the nictitating) was 9 seconds. Seven successive observations gave 10, 8, 9, 9, 9, 9, 9.2 seconds. The cava was clamped, the splanchnic stimulated and the clamp removed after stimulation had been stopped. The eye-reaction appeared in 9 seconds after removal of the clamp. Later on in the experiment, the time interval between beginning of stimulation of the splanchnic and beginning of the eye-reaction was as usual notably increased (to 12, 15, 15, 19.1, 20, and 13.1 seconds in different observations). In the meantime, repeated injections of methylene blue and adrenalin solution had been made. Atropin solution had also been instilled into the right (normal) eye to enable the circulation time to the retina to be determined.

In an experiment on a cat (whose left superior cervical ganglion had been excised 7 days previously) towards the end of the experiment the time interval of the reaction as elicited by stimulation of the splanchnic (in the thorax) had much increased (from 9.3 seconds at the beginning of the experiment to 15.8 seconds). The right splanchnic was stimulated for a minute during occlusion of the inferior cava. There were deep asphyxial respirations during which both pupils dilated, but contracted again before removal of the clamp. On removing the clamp after an occlusion lasting 63 seconds, the left pupil dilated in 15.2 seconds. Stimulation of the right splanchnic with the cava free was followed by dilatation of the left pupil in 15.8 seconds. Movement of the nictitating membrane was not signalled till 19.2 seconds.

It is particularly important to note that when such an increase in the time interval of the eye reaction elicited by stimulation of the splanchnic with the vessels open has occurred, the same increase is found when the reaction is evoked by releasing the cava when the splanchnic has been excited during its occlusion. The same factors must therefore be responsible in both cases for the greater interval after which the reaction is obtained. Of these factors slowing of the circulation can sometimes be shown to be one. Now slowing of the circulation could only affect the time-interval to exactly the same extent in the two cases if the reaction were caused by a substance moving with the blood. Other factors in the increased time-interval may be increasing sluggishness of response of the mechanisms of the eye and, as will be shown later on, diminished liberation of epinephrin by stimulation of the splanchnics.

EXPERIMENTS IN WHICH THE ARTERIAL FLOW TO THE ADRENALS WAS INTERFERED WITH

Some experiments were made with the view of determining whether the reaction could be obtained when the splanchnics were stimulated with the aorta clamped just above the diaphragm. Here, the circulation through the eyeball is of course not interfered with, and if epinephrin in sufficient quantity is liberated into the capillaries of the adrenals, and a sufficient movement of blood in the veins is still present to enable it to arrive at the heart, there is no reason why the usual response should not be elicited, even while the aorta remains occluded. When stimulation of the splanchnic was begun simultaneously with the application of the clamp an eye response was always observed, provided that the splanchnic stimulation was yielding such a response with the circulation free. Accordingly, a brief interference with the arterial flow through the glands does not abolish the secretion. In different experiments, however, there was a certain variability in the time at which the eye reactions appeared. Usually a response was obtained while the aorta still remained obstructed, and a second response after the clamp

was removed. The time interval between the beginning of stimulation and the first response, or between the removal of the clamp and the second response, is of the same order of magnitude as the time interval of the reaction with the vessels free. In other experiments no eye reaction was seen while the clamp remained on the aorta, but a response occurred (with the usual, or a somewhat longer time interval) after removal of the clamp.

Thus, in an experiment on a cat, whose left superior cervical ganglion had been removed 1 week previously, stimulation of the right splanchnic in the thorax was followed by dilatation of the pupil (and withdrawal of the nictitating membrane) of the left eye in 11.2 seconds. Five minutes later the aorta was clamped and the splanchnic stimulated for the same length of time, and with the same distance between the coils as in the previous observation. There was no effect on the eye for the 25 seconds during which the clamp was kept on, but 14 seconds after its removal the pupil dilated, and a little later the nictitating membrane moved back.

In an experiment on a dog, whose left superior cervical ganglion had been removed 6 days previously, the left splanchnic (in the thorax) was stimulated until the pupil of the left (denervated) eye began to dilate in 10.2 seconds from the beginning of stimulation. Two minutes later the left splanchnic was again stimulated, and dilatation of the pupil was obtained in 10.4 seconds. The aorta was then clamped and the splanchnic stimulated for 15 seconds with the same distance between the coils. Dilatation of the pupil occurred at 14.2 seconds from the beginning of stimulation, while the clamp was still on. The clamp was removed after an occlusion of 25 seconds, but no further change was observed in the eye. In another observation in the same animal, a few minutes later, the aorta was clamped and the splanchnic stimulated for 15 seconds. Dilatation of the pupil took place 14.6 seconds from the beginning of stimulation, while the clamp was still on. On removing the clamp, a second dilatation occurred in 9.8 seconds. The first dilatation was slight and remained so till the second carried the response on to moderate dilatation.

In an experiment¹ on a cat, to which morphin had been administered, and whose pupils remained dilated, stimulation of both splanchnics (in the thorax) for 2 seconds caused retraction of the nictitating membrane

¹ The condensed protocol is given in table 8.

and widening of the aperture of the denervated eye in 13 seconds. Two minutes later the observation was repeated, with the aorta clamped, and the eye-reactions appeared in 14.8 seconds, while the clamp was still on. After an interval of 2 minutes, stimulation of the splanchnics was again made for 5 seconds, and the eye response was elicited in 11.2 seconds, with the aorta open. The observation was repeated, the splanchnics being stimulated for the same length of time, with the aorta clamped, and the response occurred in 11.8 seconds, the clamp being still on the aorta. An observation was then made in which the aorta was clamped for 5 seconds before stimulation of the nerves was begun. It remained clamped until the eye response was obtained 11.8 seconds from the beginning of stimulation. On releasing the aorta, further retraction of the nictitating membrane occurred in 15.4 seconds from the moment of removal of the clamp. Forty-eight minutes later, stimulation of the splanchnics for 10 seconds caused a slow retraction of the nictitating in 15 seconds, with the aorta free, and in 15.4 seconds with the aorta clamped.

The explanation of the variability in the results of stimulation of the splanchnics with the aorta clamped is, we suggest, as follows: When the arterial flow is stopped for such periods as we have employed, epinephrin is still liberated into the blood vessels of the adrenals in response to stimulation of the nerves. Blood, doubtless containing some of the epinephrin, since the latent period of the secretion is quite short, continues to move out of the adrenal veins and along the cava after the clamping of the aorta, so long as the pressure remains higher in the arteries peripheral to the clamp than in the veins. As soon as this blood has reached the heart, there is, of course, no reason why it should not be carried as rapidly as before to the eyeball, and no reason why it should not produce there the characteristic reactions, provided that the quantity of epinephrin in it is sufficient. When the clamp is removed from the aorta, a residue of blood containing the balance of the liberated epinephrin is still lying in the adrenal vessels, and begins in its turn to move towards the heart. It is clear that it will depend upon the amount of epinephrin in these two fractions whether a detectable reaction shall be produced in the eye while the clamp is still on the aorta, or

only after its removal, or both after and before removal of the clamp.

The fact that as a rule the time interval of the reaction is somewhat longer when stimulation of the nerves is made with the aorta clamped than when it is made with the vessels free in all probability depends in part on this division of the epinephrin charge liberated by a given stimulation into two parts. For it can be shown that doses of adrenalin which cause, on injection, into a vein, only a minimum eye response, elicit this response after a somewhat greater interval of time than larger doses. Further, the passage of blood from the adrenals to the heart in the first few seconds after clamping the aorta must be slower than with the vessels free, although observations on the injection under minimum pressure of very small volumes of adrenalin solution into the central end of the femoral vein, with the aorta clamped, indicate that this slowing is not important in comparison with the total time interval of the reaction. Some retardation of the response after unclamping the aorta might be expected to be caused by the fact that the depleted vessels have first to be filled up by the arterial blood.

The maximum effect on the eye and the duration of the effect were in general less where the splanchnics were stimulated with the aorta clamped, than in control observations with the vessels free. The division of the liberated epinephrin into two fractions, as suggested, is a sufficient explanation, even if the total amount liberated during a brief interference with the arterial circulation remains undiminished. It is further in accordance with this explanation, that the time interval of the eye response when the splanchnics are stimulated with the inferior vena cava clamped, and the clamp then removed, agrees rather with the time interval of the response elicited with the vessels free than with the aorta clamped. This is also true of the maximum response reached for a given stimulation. Where the vena cava is clamped the epinephrin liberated by the stimulation of the nerves accumulates, of course, behind the clamp, and passes on as a single charge after removal of the clamp.

A period of 5 to 10 seconds of anemia (caused by clamping the aorta) preceding stimulation of the splanchnics, had no noticeable effect in diminishing the reaction. When the preliminary period of anemia was increased to 20 seconds, in the same animal (see table 8), stimulation of the nerves was without result in one observation, but in another observation an eye response was obtained when the splanchnics were excited after a preliminary anemia of 20 seconds. We have not hitherto made any experiments with the view of determining the precise period of anemia which will prevent a reaction. It is to be expected, of course, that it will vary to some extent in different animals, and at different times in an experiment on the same animal.

That epinephrin is actually carried in the blood of the inferior cava to the heart, while the aorta remains occluded, can be shown by injecting adrenalin solution into the central end of the femoral vein under the minimum pressure necessary to permit it to enter the vein, and in such small volume that it cannot possibly be mechanically forced along to the heart during the injection. The eye reactions are obtained after a time interval of the same order of magnitude, whether given quantities of adrenalin solution are injected with the aorta free or clamped. The interval may, indeed, be shorter with the aorta clamped. This is precisely the case when a given dose of adrenalin is injected in the form of a smaller volume of stronger solution than in the form of a larger volume of weaker solution, as is well illustrated in the first part of the protocol reproduced in table 8.

Thus, 0.02 mg. adrenalin injected (between 3.15 and 3.42 p.m.), in the form of 2 cc. of a 1: 100,000 solution, caused an eye-reaction at very much the same time interval as 0.02 mg. injected in the form of 0.5 cc. of 1: 25,000 adrenalin, with the aorta free (average time for the weaker solution 9.2 seconds; for the stronger 8.4 seconds). With the aorta clamped, the same quantity of the weaker solution gave a time of 10.8 seconds, and the stronger an average time of 7.8 seconds. While there are, of course, variable factors in such observations, which cannot all be controlled, the difference is suggestive. Later on in the experiment, when the time interval of the reaction elicited by stimulation of the splanchnic (with cava clamped) had considerably increased (to 16.6

seconds at 6.00 p.m.), the difference was even more striking. 0.1 cc. of a 1:25,000 solution of adrenalin injected with the aorta free, gave the response after an interval of 16.6 seconds (at 6.01 p.m.), the retraction of the nictitating membrane being very slow, but steadily progressing. Later on 0.2 cc. of the same solution gave a good response after an interval of 16.8 seconds (at 6.36 p.m.), while 0.2 cc. of the solution, injected with the aorta clamped, gave an interval of 11.6 seconds, and 0.1 cc. an interval of 9.8 seconds. The injection of 0.04 cc. of 1:25,000 adrenalin (with the vessels open), was followed by very slow retraction of the nictitating in 17 seconds.

These results are puzzling at first sight, but in all probability they depend upon two factors. First, the clamping of the aorta increases the blood pressure, and diminishes the circulation time in the head end of the animal. This will be especially influential in diminishing the interval at which the eye response to adrenalin injection occurs when the circulation has already deteriorated, as towards the end of a long experiment. Among other things, the action of the heart will be improved by the better filling of the coronary vessels, and the latency of the structures in the eye-ball may also be diminished by the improved circulation. Provided, then, that the adrenalin reaches the heart without undue delay, we should expect the response to occur sooner with the aorta clamped. But why should injection of a larger volume of weaker solution (with aorta clamped) not evoke the response in the same time, as the injection of a correspondingly smaller volume of the stronger solution? If the volume of the weaker solution were so great that practically the whole of it passed directly under the injection pressure into the heart, there could, of course, be no such delay as was observed. But the residual movement in the inferior cava after the aorta has been clamped may not be adequate to rapidly transport such a volume as 2 cc., while the whole or the greater part of such a volume of adrenalin solution as 0.1 to 0.5 cc. may be quickly transported to the heart. The eye reaction elicited when the greater volume was injected would be a reaction corresponding to a smaller concentration and to a smaller total quantity, than when a smaller volume of the stronger solution was injected.

EXPERIMENTS IN WHICH THE SPLANCHNICS WERE STIMULATED,
WHILE THE CIRCULATION WAS STOPPED OR SLOWED BY
INHIBITION OF THE HEART

Further evidence that the eye reaction is entirely due to a substance (epinephrin) moving in the blood from the adrenals is obtained by stopping the circulation by stimulation of the vagus.

In an experiment on a dog, the eye reaction was got in 10.2 seconds from the beginning of stimulation of the left splanchnic in the thorax. In another observation immediately thereafter, the time was 10.4 seconds. The peripheral end of the left vagus was now stimulated so as to cause complete disappearance of the pulse in the aorta. Three seconds later the left splanchnic was stimulated. Excitation of the vagus was continued for 15 seconds. The (denervated) eye reaction did not appear till 20 seconds from the beginning of stimulation of the splanchnic, or 8 seconds after stoppage of the vagus stimulation. Four minutes later the splanchnic was again stimulated, simultaneously with the vagus, the stimulation of the latter continuing for 35 seconds. No eye-reaction appeared during all this time. Three minutes thereafter stimulation of the splanchnic alone gave an eye reaction in 8.2 seconds. The vagus was again stimulated for 33 seconds so as to stop the pulse in the aorta. The splanchnic was stimulated for 15 seconds with a stronger current than in the previous observations. Eye reactions were obtained 42 seconds after the beginning of the stimulation of the splanchnic, or 11 seconds after cessation of vagus stimulation. Stimulation of the splanchnic alone now gave an eye reaction in 8.4 seconds.

Fully as convincing as the experiments in which complete stoppage of the circulation was produced, are those in which it was simply slowed by the vagus or by hemorrhage.

In an experiment on a cat (whose left superior cervical ganglion had been removed a week before), stimulation of the right splanchnic in the thorax was followed by the eye reaction in 10 seconds from the beginning of stimulation. Stimulation of the peripheral end of the right vagus and of the splanchnic was then made simultaneously. The heart was slowed but not stopped. The eye-reaction appeared in 15.2 seconds. The splanchnic was again stimulated alone, and the eye-reaction occurred in 10.2 seconds. Another observation was thereafter made with simultaneous stimulation of the vagus and splanchnic.

The eye reactions (pupil dilatation and retraction of nictitating) appeared in 15.2 seconds. When the splanchnic only was now stimulated, the reaction occurred in 11.2 seconds. Another vagus and splanchnic observation in which stimulation of the vagus was begun in advance of the splanchnic stimulation, so as to be sure that the slowing of the circulation embraced the whole time of passage of the epinephrin to the eyeball, gave the eye reactions after an interval of 15 seconds from the beginning of excitation of the splanchnic.

Later on in the same experiment the eye reaction was obtained 15.8 seconds after the commencement of stimulation of the splanchnic. Hemorrhage was now produced from the femoral artery, and the splanchnic stimulated immediately thereafter. The eye reaction did not occur till 21.6 seconds from the beginning of stimulation.

COMPARISON OF EYE REACTION ELICITED BY INJECTION OF ADRENALIN AND BY STIMULATION OF THE SPLANCHNICS.

LATENT PERIOD OF EPINEPHRIN SECRETION

Another method of showing that the eye reactions studied are caused by epinephrin liberated from the adrenals, is to inject a small amount of adrenalin into the left renal vein, or, what comes almost to the same thing for this purpose, into the central end of a femoral vein; and to determine the time from the beginning of injection to the beginning of the eye reaction. The femoral vein has the advantage over the renal that its use does not entail opening of the abdomen, or manipulation in the neighborhood of the adrenals. The circulation time from the mouth of the femoral vein, up to which the column of adrenalin solution extends before injection, will of course be somewhat greater than that from the adrenal veins. But for our purpose the difference is not important, as shown by circulation time measurements.

In an experiment, on a cat, the following results were obtained: Stimulation of the left splanchnic in the thorax gave in three successive observations, 9.4, 11.2 and 10.8 seconds as the time interval of the eye reaction. Stimulation of the right splanchnic gave 11.8 seconds, of both splanchnics together in two observations, 10.6 and 10 seconds, respectively. Injection of 1 cc. of 1:25,000 adrenalin solution in Ringer's, into the central end of the femoral vein was followed by dilatation of the

pupil in 10.6 seconds. The same amount of adrenalin was now injected while the vena cava was elamped. The pupil was unaffected till 11.2 seconds after removal of the elamp, when it dilated. Both splanchnics were now stimulated, and the eye reaction was obtained in 11.4 seconds. Injection of 0.5 cc. of 1:25,000 adrenalin into the femoral vein was followed by dilatation of the pupil in 11.2 seconds.

The quantities of adrenalin injected in this experiment were such as caused about the same reaction in the eye as the stimulation employed for the splanchnics. It is clear enough that with such quantities, the interval between the beginning of injection and the beginning of the eye reaction is approximately the same whether the reaction is elicited by adrenalin artificially introduced, or by epinephrin liberated from the glands. This indicates that the epinephrin liberated by splanchnic stimulation passes so rapidly into the veins that the latent period does not constitute a fraction of the total time interval to the commencement of the eye reaction long enough to be appreciated in such measurements. So far as these observations go, the indication is also that the material as liberated from the adrenals is, as regards the production of the reaction studied, the same as adrenalin.

On the other hand, if the quantity of adrenalin injected is much greater than is required to produce an effect upon the eye equal to that caused by splanchnic stimulation, the time interval between the beginning of the injection and the beginning of the eye reaction is reduced decidedly below that between the beginning of stimulation of the splanchnic and the commencement of the eye reaction, although it always remains, of course, greater than the circulation time to the carotid.

This is illustrated in the following experiment on a cat: 2 cc. of adrenalin (1:50,000) in methylene blue solution, was injected into the femoral vein. The methylene blue first reached the carotid in 3 seconds, and the pupil began to dilate in 5.2 seconds. In another observation, 5 minutes later, the numbers came out 3.2 seconds for the circulation time to the carotid, and 5.6 seconds for the pupil reaction. Before the first adrenalin injection, the time interval for the pupil reaction elicited by stimulation of the splanchnic was 9 seconds

in one observation, and 8 seconds in another. In an observation made 5 minutes after the second injection of adrenalin, the time interval of the eye response following splanchnic stimulation, was 8.5 seconds. The reduction of the interval, in the case of the adrenalin injection, could not be due to a change in the circulation time. It must be attributed to a reduction in the latent time of the eye mechanisms. It is probable that the time required for the blood to pass from the carotid through the capillaries of the iris to the veins, would be not much less than 2 seconds. Accordingly in this strength (and with an iris as sensitive as in this animal in the earlier part of the experiment) the adrenalin began to produce its effect, upon the pupil very soon after the first blood containing it had completely traversed the sensitive structures. Later on in the same experiment, the time interval both for splanchnic stimulation and for injection of adrenalin increased, while the circulation time to the carotid remained the same (3 seconds). This also can only be due to increasing sluggishness of the iris, with perhaps some increasing vasoconstriction in peripheral vessels including those of the eyeball.

Of course, by forcibly injecting the adrenalin solution under considerable pressure, it is possible to produce a definite diminution in the time interval of the eye response, since under these conditions, the passage of the adrenalin from the point of injection to the heart is practically instantaneous, and the pressure in the right heart may also be artificially raised, and the circulation time through the lungs correspondingly shortened. This is well illustrated in table 7, in the observations at 5.48 and 5.58 p.m., and in those between 7.12 and 7.18 p.m.

In another experiment, on a dog, the results shown in table 1 also illustrate the fact that with different doses of adrenalin different time intervals may be obtained for the (denervated) eye reaction.

In this dog (weighing 5.5 kg.), the superior cervical ganglion had been removed 6 days before the experiment. The eye-reactions were elicited with great ease by stimulation of the splanchnics and by massage of the glands; and also by very small doses of adrenalin. Exactly the opposite was the case with the cat some results from which are given in table 2. Here the eye reactions were obtained with much greater difficulty through

splanchnic stimulation, and relatively large doses of adrenalin were required to bring them out. When stimulation of the splanchnics finally became ineffective, massage of the glands yielded no result, or only a doubtful one. The epinephrin content of the glands, removed in both animals after a long experiment, was in this dog the highest which we have hitherto observed in the course of the work, while in the cat it was with one exception the lowest. The cat had brought forth a litter of kittens 13 days before the experiment, and was suckling them, and the adrenals were enlarged. It is not known whether the low epinephrin content was correlated with the parturition and lactation. The left adrenal of the cat weighed 0.317 gram, and contained 0.12 mg. epinephrin. The right adrenal weighed 0.301 gram, and also contained 0.12 mg. epinephrin.

TABLE 1

TIME	INJECTED INTO FEMORAL VEIN	PUPIL DILATATION BEGINS IN
		<i>seconds</i>
2.15	1.0 cc. Adrenalin (1: 50,000).....	10.8
2.17	0.5 cc. Adrenalin (1: 50,000).....	11.4 (less dilatation)
2.22	0.4 cc. Adrenalin (1: 50,000).....	13.0 (small effect)
2.26	2.0 cc. Adrenalin (1: 250,000).....	12.4
2.28	1.0 cc. Adrenalin (1: 250,000).....	11.2
2.30	0.5 cc. Adrenalin (1: 250,000).....	16.6

It must be stated that our estimations were almost invariably made on the adrenals of animals on which more or less prolonged experiments had been carried out. High epinephrin contents were therefore not to be expected.

The increasing time interval of the response with stimulation of the splanchnics, in the latter part of this experiment (table 2), has the same significance as increase of the time interval with small doses of adrenalin. For the pupil dilatation obtained, from successive stimulations of the nerves, became less and less, and, as already stated, when the maximum dilatation and the duration of a reaction are small, the time of onset of the reaction is delayed. Another point well brought out in this experiment was that doses of adrenalin, which, at one stage are ineffective, may

TABLE 2

Condensed Protocol of experiment on a cat, weighing 2 kg., whose left superior cervical ganglion had been removed 15 days before the experiment

TIME		PUPIL DILATATION BEGINS IN
		<i>seconds</i>
12.18	Stimulated both splanchnics for 10 seconds.....	11.4
12.31	3 cc. Adrenalin (1: 25,000).....	9.8
12.36	1 cc. Adrenalin (1: 25,000).....	11.2
12.43	1 cc. Adrenalin (1: 100,000).....	17.8 Dilatation very slight
12.45	3 cc. Adrenalin (1: 100,000).....	11.8 About same dilatation as with splanchnic stimula- tion
12.48	1 cc. Adrenalin (1: 25,000).....	12.8
12.50	1 cc. Adrenalin (1: 25,000).....	12.2
12.52	2 cc. Adrenalin (1: 25,000).....	12.4 Better dilata- tion than with 1 cc.
12.58	2 cc. Adrenalin (1: 25,000) (with cava clamped). Cava released.....	No dilatation 13.0
1.00	Both splanchnics stimulated 10 seconds.....	13.6 Dilatation small
1.03	2 cc. Adrenalin (1: 25,000).....	11.0
1.06	2 cc. Adrenalin (1: 25,000) (with cava clamped). Cava released.....	No dilatation 12.0
1.08	Both splanchnics stimulated for 10 seconds.....	15.0 Slight dilata- tion
1.27	2 cc. Adrenalin (1: 40,000).....	11.0 Dilatation prolonged
1.29	1 cc. Adrenalin (1: 40,000).....	13.8
1.30	1.4 cc. Adrenalin (1: 40,000) (with cava clamped).....	No dilatation 12.0
1.39	1 cc. Adrenalin (1: 40,000) (with cava clamped). Cava released.....	No dilatation 13.2
1.58	2 cc. Adrenalin (1: 40,000) (with aorta clamped for 14 seconds from beginning of injection)..	13.2
2.00	Both splanchnics stimulated.....	No dilatation

later on become effective; and still later may again cease to yield a response.

Thus, between 11.58 and 12.06, four observations (not reproduced in the Table) were made, in which were injected respectively 1 cc. of adrenalin (1:100,000), 2 cc. of adrenalin (1:100,000), 2 cc. of adrenalin (1:50,000) and 3 cc. of the 1:50,000 solution without effect on the eye. Between 1.16 and 1.19 p.m., three injections, two respectively of 2 cc., and one of 3 cc. of 1:100,000 adrenalin were without result. As shown in table 2, at 12.43 p.m., 1 cc. of the same solution, and at 12.45 3 cc., gave positive results. Naturally, variations in the anesthesia may play a part, in this variability of the response, and the point is mentioned merely to illustrate the fact that numerous controls are required in such observations.

It has been already stated that in prolonged experiments, the increase in the time interval between the beginning of stimulation of the splanchnics, and the beginning of the eye reaction, which is observed towards the end of the experiment, may be shown by measurements of the circulation time to be partly due to slowing of the circulation. Another factor, however, is involved, namely, the increase in the latency of the iris response, after the epinephrin has reached the eye. In the time interval, three factors are plainly concerned: first, the latent period of the adrenal gland, that is, the time that elapses between the beginning of an effective stimulation and the appearance of epinephrin in the blood; (2) the circulation time from the adrenal to the eye ball; (3) the latent period of the structures in the eye ball which yield the reaction. The observations already mentioned show that the first factor constitutes a very small part of the whole time interval. It may vary with the strength of the stimulus to some extent, but this cannot easily be determined, because the moment at which the eye reaction can just be seen to begin also depends somewhat on the amount of epinephrin liberated. In comparison with the time required for its transportation to the eye, and with the lost time there before the sensitive structures respond, the latent period of the secretion may be considered negligibly small.

MINIMUM DURATION OF EFFECTIVE STIMULATION OF THE
SPLANCHNICS

The minimum time of stimulation necessary to produce the liberation of an amount of epinephrin just sufficient to cause an appreciable reaction, is also very short. It varies, of course, with the strength of stimulation. With a stimulus (faradic) strong enough to be sharply felt on the tongue, one-half of a second of stimulation sufficed to give a reaction. With increase in the time of stimulation, the maximum amount of dilatation of the pupil obtained and the duration of the dilatation increased, and retraction of the nictitating membrane, which was not got with the short periods of stimulation, was seen. The maximum effect in both reactions was reached more speedily with increas-

TABLE 3

TIME	DURATION OF STIMULUS	BEGINNING OF PUPIL DILATATION	NICTITATING RETRACTED
	<i>seconds</i>	<i>seconds</i>	
10.44	1	8.0	+
10.45	$\frac{1}{2}$	9.0 (slight)	-
10.47	3	9.0	+
10.48	5	9.0	+
10.50	10	9.0	+
10.52	15	9.2	+

ing time of stimulation. In an animal in which a reaction is being easily elicited the total time interval, between the beginning of stimulation and the commencement of the eye reaction, may be approximately the same for the longer as for the shorter periods of stimulation. This is well shown in tables 3 and 4, taken from experiments on two cats. Where the eye reaction (or the adrenal secretion) is becoming exhausted in the course of a long experiment the minimum time of stimulation needed to produce an effect may be greater than earlier in the experiment. When this is the case, the time interval to the beginning of the eye reaction may be diminished as the duration of the stimulus is increased, as is shown in table 5, taken from the same experiment as table 4.

COMPARISON OF CIRCULATION TIME FROM ADRENALS TO EYEBALL,
WITH TIME AT WHICH THE EYE REACTION APPEARS

The circulation time from the level of the adrenals to the carotid and to the eyeball was determined in a number of experiments by the methylene blue method (8), in order to verify the assumption that the time interval of the eye reaction was of the order of magnitude appropriate to a reaction elicited by a substance moving from the adrenals to the eye with the average speed of the blood. In every case the time required for blood to

TABLE 4

TIME	DURATION OF STIMULUS	BEGINNING OF PUPIL DILATATION	NICTITATING RETRACTED
	<i>seconds</i>	<i>seconds</i>	
11.32	5	9.6	+
11.35	$\frac{1}{3}$	9.6 (very slight)	—
11.37	1	10.2	—
11.38	3	10.0	in 11 seconds

TABLE 5

TIME	DURATION OF STIMULUS	BEGINNING OF PUPIL DILATATION	NICTITATING RETRACTED
	<i>seconds</i>	<i>seconds</i>	
12.35	15	13.4	13.4
12.38	$\frac{1}{3}$	No dilatation	—
12.40	1	No dilatation	—
12.42	3	No dilatation	—
12.44	5	18.6 (very slight)	—
12.46	10	15.0 (very slight)	—

pass to the eye or even through it, was found to be less than the time interval at which the reaction occurred when elicited by stimulation of the splanchnics. A margin was always left for the time necessary for the reaction to be developed to the point at which it could be detected after the epinephrin had reached the sensitive structures.

In an experiment, on a cat, 1.5 cc. of a solution of methylene blue made with Ringer's solution, was injected into the left renal vein.

The right splanchnic in the thorax was stimulated from the beginning

of the injection, the stimulation being continued for 5 seconds. The methylene blue was first observed in the carotid at 4.2 seconds, and the pupil began to dilate at 10.2 seconds from the beginning of injection. 1.5 cc. of 1:50,000 adrenalin in the same solution of methylene blue was then injected into the femoral vein. The color was seen in the carotid at 6 seconds, and dilation of the pupil occurred at 10.2 seconds. An injection of 2 cc. of the adrenalin-methylene blue solution was then made, and the color was observed in the carotid at 5.2 seconds, the dilatation of the pupil beginning at 9.2 seconds. Later on in the experiment, when the animal was in distinctly worse condition, stimulation of the right splanchnic (for 15 seconds) gave dilatation of the pupil and retraction of the nictitating on the denervated side in 13.2 seconds from the beginning of stimulation. Injection was then made into a femoral vein of 2 cc. of 1:50,000 adrenalin in methylene blue solution. Dilatation of the pupil occurred in 12.2 seconds. In a later observation 4 cc. of the adrenalin methylene blue solution was injected. The blue was seen with the ophthalmoscope in the retinal vessels of the normal eye at 11.4 seconds, and the pupil dilatation occurred in the denervated eye at 14.4 seconds. The normal eye was dilated with atropia. When this observation was repeated, 7 minutes later, the color was detected in the retina in 10.6 seconds, and dilatation of the pupil took place in 12.2 seconds.

The protocol in table 6 further illustrates experiments in which simultaneous observations of the eye reaction caused by adrenalin, and the circulation time, as determined by the methylene blue method, were made.

The circulation time from the left renal vein or the femoral vein to the carotid artery corresponds to a little less than the time required for the first of the epinephrin liberated from the adrenal to reach the iris. Probably something like 2 seconds must be added for the circulation time through the iris capillaries. (It was found by one of us (9) that the time from the central artery to the central vein of the retina in chloralized rabbits was from 1.7 to 1.9 seconds.) Even allowing for this, it is evident that it takes some sensible time after epinephrin has traversed the sensitive structures in the eyeball before a reaction is seen, at least under the conditions of our experiments.

TABLE 6

*Condensed protocol of experiment on a cat. Left superior cervical ganglion removed
16 days before the experiment*

TIME		SEEN IN CAROTID AFTER	PUPIL REACTION
		<i>seconds</i>	<i>seconds</i>
10.20	Urethane 1 gram.....		
11.00	Right splanchnic ligated and cut in thorax..		
11.02	Stimulated splanchnic.....		9.0
11.04	Inferior vena cava clamped; splanchnic stimulated for 15 seconds. No effect on the pupils while the cava was clamped; after 30 seconds cava unclamped, and 9 seconds later pupil dilated.....		9.0
11.08	Repeated observation made at 11.04.....		10.0
11.10	Stimulated splanchnic.....		8.0
11.40	Injected 2 cc. methylene blue into renal vein.	4.0	
11.42	Injected 1.5 cc. methylene blue into renal vein.....	3.8	
11.44	Injected 1.5 cc. methylene blue into renal vein.....	3.0	
11.46	Injected 1.5 cc. " " " "	4.0	
11.48	Injected 1.5 cc. " " " "	3.2	
11.59	Injected 2 cc. of 1:50,000 adrenalin in methylen blue.....	3.0	5.2
12.02	Repeated last observation.....	3.2	5.6
12.07	Stimulated splanchnic.....		8.5
12.10	Clamped cava for 32 seconds. Stimulated splanchnic 15 seconds from the time of clamping.....		No dilatation
	Removed clamp.....		10.0
12.15	Clamped cava. Injected 2 cc. adrenalin- methylen blue into renal vein.....		No dilatation
	Removed clamp after 24 seconds.....		7.0
12.20	Repeated last observation. Cava clamped 37 seconds.....		No dilatation
	Removed clamp. Methylene blue seen in carotid in.....	3.0	9.5
12.25	Injected 2 cc. adrenalin-methylen blue into renal vein.....	3.3	8.2
12.40	Stimulated splanchnic for 15 seconds.....		19.0
12.42	Stimulated splanchnic with stronger stimulus.		No effect
12.56	Massaged left adrenal.....		No effect

EXPERIMENTS IN WHICH THE ARTERIAL FLOW TO THE EYEBALL
WAS INTERFERED WITH

The experiments on occlusion of the venous path from the adrenals were supplemented by observations in which the arterial path to the eyeball was interfered with. The circulation in an eyeball is not completely stopped when the corresponding common carotid is occluded. If, then, the response to stimulation of the peripheral end of the splanchnic is solely due to a substance carried in the blood, it is obvious that it will depend upon the amount of blood reaching the eye by other routes, upon the amount of epinephrin liberated by a given stimulation of the splanchnics and upon the sensitiveness of the reacting structures to epinephrin, whether the reaction yielded by splanchnic stimulation shall be entirely suppressed or merely postponed, and perhaps weakened, by occlusion of one or of both carotids. It ought, indeed, to be merely a matter of properly choosing the duration and strength of stimulation, and the degree of interference with the arterial path to the eye, to bring about complete suppression of the eye reaction or to permit it to be elicited. Further, the details of the eye response to splanchnic stimulation, when the flow of blood to the eye is more or less interrupted, should be capable of being imitated by injecting greater or smaller quantities of adrenalin into a vein. This is fully as severe a test as the occlusion of the venous path from the adrenals. For a greater variety of result is possible. Yet all the variations must agree with the assumption that the effective factor, in bringing about the eye response, is circulating epinephrin. If, for instance, it were found that with one or both carotids clamped the eye reaction followed sooner on stimulation of the splanchnic than with the vessels free, this could not be reconciled with the assumption aforesaid, for it must take longer for the epinephrin to reach the eye ball by less direct routes, and less of it will arrive at the sensitive structures.

As will be seen from the condensed protocol of the experiment reproduced in table 7, the results of clamping the ipsilateral or the contralateral carotid, or of both, with varying intensity and

duration of stimulation of the splanchnic, are invariably what they ought to be if the eye reaction is due to a substance carried in the blood. Also, each result can be imitated by injecting different quantities of adrenalin solution into the femoral vein with one or both carotids clamped. The occlusion of the carotid has the slight disadvantage from the point of view of technique, that it causes some dilatation of the pupil itself. This, however, when the splanchnic stimulation or adrenalin injection is made immediately after clamping, is not a serious drawback, and the beginning of the more sudden dilatation of the pupil due to epinephrin is easily recognized when superposed upon the slow dilatation due to interference with the circulation.

It will be seen that in this animal, in which the eye response was elicited by stimulation of the splanchnic with great ease, it was necessary to reduce the time of excitation even when only one splanchnic was stimulated to 2 seconds, and to reduce the strength of the stimulus in order to abolish the reaction completely when both carotids were clamped. On releasing the carotids, a response was not in general obtained comparable to the response observed when the vena cava is released after a period of stimulation. It is clear that this ought to be the case, because the epinephrin instead of being dammed back behind the clamp, as when the cava is occluded, must have passed for the most part into the general circulation during occlusion of the carotid. It is of course possible that when unusually large quantities of epinephrin are liberated, an after response may be obtained on releasing the carotids. With a shorter distance between the coils, and the same time of stimulation, or with a longer time of stimulation and the same distance between the coils, so much more epinephrin was liberated from the adrenals that enough of it found its way to the denervated eye, even with both carotids clamped, to produce after a much lengthened time interval the characteristic reaction. When only one carotid was clamped, occlusion of the ipsilateral artery had a much greater effect than clamping of the contralateral artery, in lengthening the time interval. The fact that doses of adrenalin could be determined which, when injected into a vein, behaved as

TABLE 7

Condensed protocol of experiment on a cat weighing 3 kg., whose left superior cervical ganglion had been excised 9 days before. The numbers in brackets are the distances between the coils in stimulating.

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
1.00	2.5 grams urethane.....		
1.30	Ether.....		
2.15	Left splanchnic ligated, divided and iso- lated in thorax.....		
2.30	Left splanchnic (stimulated) 9.8 seconds (coils at 12 cm.).....	9.8	+
2.33	Left splanchnic (stimulated) 10 seconds (coils at 12 cm.) (with left carotid clamped).....	12.8	+
2.35	Left carotid clamped for 10 seconds (no effect on eye).....		
2.39	Left splanchnic stimulated for 10 seconds (8.5 cm.).....	10.2	+
2.41	Left splanchnic stimulated for 6.8 sec- onds (8.5 cm.).....	6.8	+
2.43	Left splanchnic stimulated for 5 seconds (9 cm.).....	7.0	+
2.45	Left splanchnic stimulated for 5 seconds (9 cm.) (left carotid clamped).....	8.0	
2.48	Left splanchnic stimulated for 5 seconds (10 cm.).....	8.6	
2.49	Left splanchnic stimulated for 5 seconds (12 cm.).....	6.8 (moderate)	
2.51	Left splanchnic stimulated for 2 seconds (12 cm.).....	7.2 (moderate)	+
3.02	Left splanchnic stimulated for 1 second (12 cm.) (left carotid clamped).....	10.8 (moderate)	+
3.05	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No eff. at 20	-
3.08	Left splanchnic stimulated for 5 seconds (12 cm.).....	7.2	+
3.10	Left splanchnic stimulated for 5 seconds (12 cm.) (both carotids clamped).....	12.4	+
3.14	Left splanchnic stimulated for 5 seconds (12 cm.).....	7.2	+
3.16	Left splanchnic stimulated for 2 seconds (12 cm.).....	10.2	+
3.23	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No react. in 25	

TABLE 7—Continued

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
3.26	Left splanchnic stimulated for 2 seconds (12 cm.) (left carotid clamped).....	16.2 (moderate)	—
3.30	Left splanchnic stimulated for 2 seconds (12 cm.).....	7.8	+
3.32	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No react. in 30*	
3.35	Clamped both carotids for 25 seconds†...		
3.44	Left splanchnic stimulated for 10 seconds (12 cm.) (both carotids clamped)††.....	12.4	+
3.49	Left splanchnic stimulated for 8.6 seconds (12 cm.).....	8.6	+
3.51	Left splanchnic stimulated for 10 seconds (12 cm.) (left carotid clamped).....	10.2	+
3.55	Left splanchnic stimulated for 10 seconds (12 cm.) (both carotids clamped).....	15.6	+
	No reaction on releasing		
4.00	Left splanchnic stimulated for 5 seconds (12 cm.) (both carotids clamped).....	15.8 (moderate)	—
4.03	Left splanchnic stimulated for 5 seconds (12 cm.) (left carotid clamped).....	No effect	
4.06	Left splanchnic stimulated for 5 seconds (12 cm.).....	8.4	+
4.08	Left splanchnic stimulated for 5 seconds (12 cm.) (left carotid clamped).....	11.0	+
4.09	Left splanchnic stimulated for 5 seconds (12 cm.) (right carotid clamped).....	9.2	+
4.12	Left splanchnic stimulated for 2 seconds (12 cm.).....	7.6	+
4.16	Left splanchnic stimulated for 2 seconds (12 c,m.) (both carotids clamped).....	No effect	
4.19	Left splanchnic stimulated for 2 seconds (12 cm.) (left carotid clamped).....	13.8 (moderate)	+
4.27	Left splanchnic stimulated for 2 seconds (12 cm.) (right carotid clamped).....	9.4	
4.47	1 cc. (1: 100,000) adrenalin into femoral vein.....	10.6	
5.07	2 cc. (1: 25,000) adrenalin into femoral vein.....	7.8	+
5.12	1 cc. (1: 12,500) adrenalin into femoral vein.....	7.4	+
5.14	1 cc. (1: 12,500) adrenalin (both carotids clamped).....	11.2	+

TABLE 7—Continued

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
5.15	0.5 cc. (1:12,500) adrenalin (both carotids clamped).....	16.6	+
5.21	0.3 cc. (1:12,500) adrenalin.....	12.8	+
5.25	0.3 cc. (1:12,500) adrenalin (both carotids clamped).....	20.0	+
5.30	0.5 cc. (1:25,000) adrenalin (both carotids clamped).....	17.2	+
5.35	Left splanchnic stimulated for 2 seconds (12 cm.).....	6.0	+
5.37	Left splanchnic stimulated for 2 seconds (12 cm.).....	6.0	+
5.40	Left splanchnic stimulated for 5 seconds (12 cm.).....	6.0	+
5.43	Left splanchnic stimulated for 5 seconds (12 cm.) (both carotids clamped).....	15.0	+
5.45	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No reaction	
5.48	0.7 cc. (1:25,000) adrenalin (slowly injected).....	11.2	+
5.58	0.6 cc. (1:25,000) adrenalin (injected faster).....	8.6	+
6.00	1.2 cc. (1:25,000) adrenalin (injected faster).....	6.0	+
6.02	1.2 cc. (1:25,000) adrenalin (both carotids clamped).....	14.2 (moderate)	+
6.04	0.25 cc. (1:25,000) adrenalin (both carotids clamped).....	No reaction	
6.20	Left splanchnic stimulated for 6.2 seconds (12 cm.).....	6.2	+
6.48	0.3 cc. (1:25,000) adrenalin.....	12.0	+
6.50	0.25 cc. (1:25,000) adrenalin.....	12.0	+
6.53	0.3 cc. (1:25,000) adrenalin (both carotids clamped).....	No reaction	
6.54	0.25 cc. (1:25,000) adrenalin (left carotid clamped).....	15.2	+
6.55	0.3 cc. (1:25,000) adrenalin (right carotid-clamped).....	11.6	+
6.56	0.15 cc. (1:25,000) adrenalin (left carotid clamped).....	17.0 (moderate)	+
6.58	0.15 cc. (1:25,000) adrenalin.....	13.4	+
7.00	0.15 cc. (1:25,000) adrenalin (both carotids clamped).....	No reaction	

TABLE 7—Continued

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
7.02	0.15 cc. (1: 25,000) adrenalin.....†	10.2	+
7.03	Right splanchnic divided isolated.....		
7.06	Right splanchnic stimulated for 8.2 sec- onds (9 cm.).....	8.2	+
7.07	Right splanchnic stimulated for 8.2 sec- onds (9 cm.).....	9.0	+
7.09	Right splanchnic stimulated for 10 sec- onds (12 cm.).....	13.0	+
7.10	Right splanchnic stimulated for 5 sec- onds (12 cm.).....	12.0 (moderate)	+
7.11	Right splanchnic stimulated for 5 sec- onds (9 cm.).....	10.0 (moderate)	+
7.12	0.8 cc. (1: 25,000) adrenalin (injected slowly).....	11.0	+
7.13	1.0 cc. (1: 25,000) adrenalin (injected slowly).....	11.0	+
7.15	1.0 cc. (1: 25,000) adrenalin (injected rapidly under pressure).....	8.4	+
7.18	2.0 cc. (1: 25,000) adrenalin (injected rapidly under pressure).....	7.4	+

Left adrenal weighed 0.214 gm. and contained 0.12 mg. epinephrin.

Right adrenal weighed 0.203 gm. and contained a trace of epinephrin (less than 0.02 mg.).

* When carotids were clamped, gradual dilatation of both pupils occurred, the left going on to almost full dilatation. On removing clamps, left pupil kept on dilating to full, and the nictitating membrane retracted at 12.4 seconds after release of carotids.

† Both pupils gradually dilated, left more than right. After release contraction of both pupils, right contracting immediately and left gradually. The left nictitating retracted 16.4 seconds after release.

†† Removed clamp after 35 seconds. The same effects were observed as is in observation of 3.35, nictating retracting 14.1 seconds after release. The effects observed when carotids were released after occlusion for periods of such length were invariably the same, and reference to them is omitted in the rest of the protocol.

regards the eye response evoked during interference with the arterial supply of the eye ball, precisely in the same way as stimulation of the splanchnic, absolutely clinches the proof that the nerve exerts its effect by liberating epinephrin.

The amount of adrenalin necessary to reproduce approximately

the effect of a stimulation of the left splanchnic for 2 to 5 seconds was about 1 cc. of a 1:25,000 solution, i.e., 0.04 mg. An assay of the adrenalin used showed that it was 10 per cent under its nominal strength. Even assuming that the necessary quantity was only 0.02 mg., the large number of successful stimulations (at least 20), if each liberated 0.02 mg. of epinephrin, would imply a very large initial store of epinephrin in the left adrenal, (higher indeed than Elliot found in any cat), if all the liberated epinephrin came from the store. Add to this, that some of the stimulations were longer than 5 seconds, and we know that the reaction and therefore the amount of epinephrin set free increases up to a certain point with duration of the stimulation. Further, there is scarcely any doubt that the eye reactions could have been elicited a much larger number of times by stimulation of the nerves, had it been the object of the experiment to determine the maximum number of successful excitations. It is suggestive in this connection that the extremely low content of epinephrin in the right adrenal, accounted for, in accordance with Elliot's work, by the corresponding splanchnic nerve having remained intact during an anaesthesia of 6 hours duration (a good deal of ether being needed), coincided with a considerable power of the right splanchnic to liberate epinephrin in response to electrical stimulation.

The distinct shortening of the time interval between the beginning of stimulation of the left splanchnic and the beginning of the eye response seen in the observations commencing at 5.45 p.m. is probably connected with improvement in the circulation due to repeated injections of adrenalin and also of the Ringer's solution in which it was dissolved.

We take the opportunity to point out here that the denervated eye reactions not only constitute an extraordinarily sensitive test for small quantities of epinephrin circulating in the blood, but also afford the means of studying certain questions connected with the mass-movement of the blood, and especially with the collateral circulation when large vessels are blocked. The demonstration of the onward flow of blood in the inferior cava, after occlusion of the aorta, has already been referred to.

As already stated, the eye response in this animal was very easy to elicit by stimulation of the splanchnic, and very difficult to exhaust. In consequence, it was no doubt more difficult to abolish it by occlusion of the carotids than in animals in which a smaller response was obtained. The cat was exceptionally large, and in large animals the collateral circulation may be expected to be freer than in small, as witness the difference in the effect of ligating the four cerebral arteries in dogs on the one hand, and in cats and rabbits on the other.

Also the thyroids were much enlarged. The clips were put on the carotids below the level of the thyroids and a freer collateral circulation than normal may have existed distal to the clips. It may be predicted, that in different animals the effect of clamping one or both carotids, on the eye response, will vary considerably with the excitability of the epinephrin-liberating mechanism, and with the abundance of the collateral circulation. As a matter of fact, in another cat, whose response to splanchnic stimulation had never been as good as in the case just described, and had been to a certain extent exhausted by the time the observations on occlusion of the carotids were made, it was very easy to abolish the response by clamping the ipsilateral carotid alone.

In this animal, whose superior cervical ganglion had been removed 8 days before, the left carotid was clamped during stimulation of the peripheral end of the left splanchnic nerve. No eye reaction was obtained, either during the occlusion or after the removal of the clamp, although stimulation of the splanchnic was effective in control observations with the carotid free. For example, in one observation, stimulation of the splanchnic was followed by full dilatation of the left pupil in 10 seconds from the beginning of stimulation. The pupil returned to normal in 1 minute. Four minutes later the carotid was clamped, and the splanchnic stimulated for 25 seconds, without result on the denervated eye. Stimulation was then stopped and the clamp removed 15 seconds later. No eye effect was obtained after removal of the clamp.

MASSAGE OF THE ADRENALS

It was shown by one of us (10) that when the adrenal (in the dog) was massaged, a quantity of epinephrin, easily detectable by the intestine and uterus segment reaction, was liberated into the adrenal vein. The concentration of epinephrin in blood collected from the vein, in one experiment, was estimated at 1:500,000. The reactions of the denervated eye are also obtained (in cats and dogs) when one or both adrenals are massaged after section of the corresponding or of both splanchnics. The time interval between the beginning of massage and the beginning of the eye reaction, when massage gives a good reaction, is the same, within the limits of error of our observations, as the interval when the eye reaction is elicited by stimulation of the splanchnic. In an animal in which splanchnic stimulation is causing a good eye response, even slight and momentary massage of the corresponding adrenal may be effective. The reaction is therefore due to the setting free of epinephrin just as when it follows stimulation of the splanchnics.

That the latent period of the liberation of epinephrin by massage is very short, as in the case of its liberation by stimulation of the splanchnic, is indicated by a comparison of the time interval at which the eye reactions occur with the interval after injection of adrenalin. For example, in an experiment on a dog the right adrenal was massaged and dilatation of the pupil occurred in the denervated eye in 11.2 seconds from the beginning of massage. 2.5 cc. of a 1:50,000 solution of adrenalin in Ringer's was now injected into the central end of the femoral vein, and dilatation of the pupil, which soon became maximal, was observed at 11.6 seconds.

The question may be asked whether the effect of massage is not really a mechanical stimulation of the nerve fibres in the glands. That it is something more is indicated by the fact that when after repeated stimulation, excitation of a splanchnic nerve has ceased to be followed by an eye reaction, a good reaction may still be obtained by massage of the gland. Ultimately, this fails also, and at a time when the gland still contains epinephrin, as determined by quantitative estimation after its removal.

For example, in an experiment on a cat (anesthetized with ether alone), the left splanchnic was stimulated before cutting it. The eye reaction was readily obtained (in 10, 11 and 10 seconds in three successive observation, in which the nerve was stimulated till the reaction appeared). After section of the splanchnic, stimulation of its peripheral end was followed in 11 seconds by dilatation of the pupil and retraction of the nictitating. The nerve was then stimulated in seven further observations for periods varying from 15 to 22 seconds. No effect was now produced on the eye. The electrodes were then applied to the left adrenal gland directly for 37 seconds. There was no response by the pupil or nictitating. Immediately thereafter massage of the left adrenal was performed, until a response was obtained in 12 seconds from the beginning of the massage. The eye response was maximal, and the pupil remained well dilated for more than 5 minutes, indicating that the massage had liberated a considerable amount of epinephrin. Five minutes after the first massage, the gland was again massaged for 30 seconds, without causing any further dilatation of the pupil, which had not at this time diminished to its original size. Three minutes later the splanchnic was stimulated for 18 seconds without effect. Massage of the gland was repeated in 3 minutes, till dilatation of the pupil occurred in 15 seconds from the beginning of the massage. The dilatation persisted for 10 seconds. Finally, the splanchnic was stimulated for 23 seconds without evoking any eye response.

In an experiment on a dog, the left splanchnic (in the thorax) had been stimulated 13 times, stimulation being in each case followed by good eye reactions. The average time of stimulation was 15 seconds. The time interval at which the eye-response followed the beginning of stimulation ranged from 8.2 to 11.2 seconds at different stages in the experiment. Two minutes before the effect of massage of the adrenals was tested, stimulation of the left splanchnic caused dilatation of the pupil in 8.4 seconds. A brief massage of the left adrenal then produced an eye response in 8.2 seconds. The dilatation of the pupil was good, and greater than that caused by the previous stimulation of the nerve. Five minutes later the left adrenal was massaged for 2 seconds; no effect was caused on the eye. After an interval of 2 minutes, the left adrenal was strongly massaged for 5 seconds, and dilatation of the pupil was seen 18.4 seconds after the beginning of the massage. The pupil, however, was still dilated from the first massage. One minute later the gland was massaged strongly, and full dilatation of the pupil was obtained in 22.4 seconds. The massage was still continued with

the object of exhausting the epinephrin store. The dilatation lasted $3\frac{1}{2}$ minutes. The left splanchnic was then stimulated for 20 seconds without effect on the eye. Massage of the left adrenal now caused a very slight dilatation of the pupil after 31.8 seconds. The right splanchnic (in the thorax) was now stimulated twice for 20 seconds without effect. Massage of the right adrenal was then performed, and dilatation of the pupil occurred 11.2 seconds from the beginning of the massage.

It will be observed that massage of the right unexhausted adrenal caused a pupil reaction after a time interval of the same order of magnitude as was obtained earlier in the experiment, while the animal was in good condition, by stimulating the splanchnic; and also of very much the same length as the time interval of the reaction elicited in the first massage of the left adrenal. The conclusion can scarcely be avoided that the reason for the extraordinary increase in the length of the interval in the later massage observations on the left adrenal is due to the fact that very little epinephrin could now be liberated by massage. It therefore took a considerable time to accumulate such an effect on the pupil as comes within the limits of observation.

The left adrenal in this dog weighed 0.376 gram and contained 0.24 mg. of epinephrin. The right adrenal weighed 0.360 gram and contained 0.42 mg. of epinephrin. The difference is striking, particularly if we reflect that the right splanchnic remained intact much longer than the left, and according to Elliott, exhaustion of the epinephrin store proceeds rapidly in an anesthetized animal, in the gland whose splanchnic supply has not been divided, whereas little if any effect is produced by prolonged stimulation of the cut splanchnic nerve. Accordingly, it seems highly probable that the difference in the epinephrin content of the two glands in this experiment represents the epinephrin liberated by the vigorous massage of the left adrenal, or a portion of this difference. The great dilatation of the pupil of the denervated eye occasioned by this massage supports the idea that the amount of epinephrin so liberated was substantial.

After a gland has failed to yield an eye reaction either by

stimulation of the splanchnic or by massage, a reaction may again be obtained from it after a period of rest. But so far as our experiments on this point go, the power to respond is never so great as at first and is soon again lost.

RELATION OF THE EPINEPHRIN STORE IN THE ADRENAL TO THE
EPINEPHRIN LIBERATED BY SPLANCHNIC STIMULATION

We have tried to determine whether there is any relation between the stock of epinephrin in an adrenal gland and the readiness with which epinephrin is given off to the blood (as tested, for example, by the minimum length of stimulation of a given strength which will yield an appreciable reaction) or the total amount of epinephrin which can be so given off in response to splanchnic stimulation or massage. The details of our observations on this point will be omitted for the present. While, as has been already mentioned, good eye reactions have been seen in animals whose adrenals at the end of the experiment were still found relatively rich in epinephrin, and poor eye reactions in animals whose adrenals at the end of the experiment contained only a small epinephrin store, we have also seen perfectly good reactions, and have been able to elicit them frequently over long periods, in animals whose adrenals when finally excised were found poor in epinephrin. It does not, at present at any rate, appear that glands with a relatively low epinephrin content necessarily give a poorer response, as tested by the eye reactions, or one more easily exhausted, than glands with a relatively high content. This statement is based merely upon the routine examination of the glands, after the experiments were finished, by the method of Folin, Cannon and Denis (1). The animals had, of course, been anesthetized for a considerable time. The epinephrin content may therefore be assumed to have been diminished to some extent. We tried in one case to bring about a high degree of exhaustion by a prolonged period of "frightening" a cat by a dog just before the experiment. But the epinephrin content was found to be fully as high as in cats in which precautions has been taken to reduce their emotional disturbance

and struggle to the minimum. The eye reaction to stimulation of the splanchnic was quite as good, and could be obtained quite as often without exhaustion, as in the run of our experiments. There is no a priori reason to assume that the epinephrin liberated into the blood in response to stimulation of the nerves must necessarily come entirely from the store already present in the glands. If it does come entirely from this store, it is not easy to understand Elliott's result, that stimulation of the splanchnic fails to cause any reduction in the epinephrin content of the corresponding adrenal. For nothing is more certain, than that epinephrin is given off into the adrenal vein blood during such stimulation. While it may be true that we cannot definitely assay this quantity, by comparing the eye response evoked by splanchnic stimulation with the quantity of injected adrenalin necessary to elicit the same amount of response, since we do not know for certain that the epinephrin as it passes into the circulation from the adrenals is quite the same thing as the adrenalin which we introduce from a burette, yet there is no doubt that when in the course of an experiment a splanchnic has been stimulated 15, 20 or even 50 times, each time evoking a definite eye reaction, a total amount of epinephrin must have passed from the gland large enough to be reflected in a diminution of the final content of that gland, unless in the meantime the epinephrin store was being recruited.

In a cat, which had received urethane the day before the experiment and morphine three hours before the experiment and which was then anaesthetized with urethane and ether, a very low content of epinephrin was found in the adrenals at the end of the experiment (see condensed protocol in table 8). The glands, it is true, had been massaged shortly before the animal was killed, but had yielded little epinephrin to the blood as judged by the eye reaction elicited by the massage. There is accordingly every reason to believe that the content of epinephrin was small throughout the whole or the greater part of the experiment. If the maximal dilatation of the pupil, not only that of the highly sensitive denervated eye but the other also, induced by the morphine and persisting for the $7\frac{1}{2}$ hours for which the cat was

observed, was maintained by epinephrin from the adrenal store, it is not conceivable that at any time after observations on stimulation of the splanchnics were begun the store could have been great. Yet such reactions as were still available (retraction of the nictitating and widening of the aperture) were readily and repeatedly evoked through stimulation of the peripheral ends of the nerves. There is no doubt that many more successful stimulations could have been made. On the assumption that each of these required the liberation of an amount of epinephrin equal to the amount of adrenalin which had to be artificially introduced in order to give a similar reaction, the question again arises, as in the experiment given in table 7, whether the whole of this could possibly have come from the stored epinephrin, or whether epinephrin liberated in response to splanchnic stimulation was formed at the moment.

In another cat, a young animal weighing 1.5 kg., the left splanchnic (in the thorax) was stimulated 52 times in the course of 4 hours. No doubt a larger number of successful stimulations could have been obtained. The amount of adrenalin hydrochloride which had to be injected into the femoral vein to yield a reaction equal to the average in the splanchnic observations was at least 0.008 mg. This would be equivalent to, say 0.4 mg. for the total number of reactions. The left splanchnic had been cut $1\frac{1}{4}$ hours after the administration of urethane, the right 37 minutes later. The right splanchnic was stimulated only 3 or 4 times, always with a positive result. The last stimulation of the right splanchnic, at the end of the experiment gave as good a reaction as those made immediately after its isolation. Both adrenals were small. The right weighed 0.104 gm. and contained 0.10 mg. of epinephrin. The left adrenal weighed 0.097 gm. and contained 0.14 of epinephrin. The small content in each case is probably due to the exhaustion of the store under the anaesthesia before the nerves were divided. The right gland, whose nerve remained longer intact, has naturally a somewhat smaller content.

But it is certainly a striking fact that the gland which had liberated enough epinephrin to cause a definite eye reaction more than fifty times² (an effect equal to that produced by 0.4 mg.

²We have since had more than 300 successful stimulations in a cat.

TABLE 8

Condensed protocol of experiment on a cat weighing 1.5 kg. Left superior cervical ganglion excised four days previously. On the day before the experiment, the animal received 1 gram urethane and was allowed to recover.

TIME		EYE-REACTION AFTER
		seconds
11.00	20 mg. morphin subcutaneously.....	
2.00	1 gram urethane, by stomach tube.....	
2.15	Etherized. Both splanchnics ligated in thorax and cut.....	
2.50	Left splanchnic stimulated.....	No effect
2.55	Both splanchnics stimulated 10 seconds.....	10.6
2.58	Both splanchnics stimulated 8.6 seconds.....	8.6
3.01	Both splanchnics stimulated (weaker current).....	9.6
3.04	Both splanchnics stimulated (weaker current).....	8.0
3.15	2 cc. adrenalin (1: 100,000) in femoral vein.....	8.6
3.25	0.5 cc. adrenalin (1: 25,000).....	8.4
3.29	2 cc. adrenalin (1: 100,000).....	9.8
3.36	Both splanchnics stimulated for 8 seconds.....	8.0
3.42	2 cc. adrenalin (1: 100,000).....	8.6
3.52	2 cc. adrenalin (1: 100,000) (with aorta clamped for 10 seconds).....	10.8
3.58	0.5 cc. adrenalin (1: 25,000) with aorta clamped for 10 seconds).....	7.4
4.12	0.5 cc. adrenalin (1: 25,000) (with aorta clamped)..	6.8
4.25	0.5 cc. adrenalin (1: 25,000) (with aorta clamped)..	9.2
4.30	Both splanchnics stimulated for 9.2 seconds.....	9.2
4.35	Both splanchnics stimulated for 10 seconds (with aorta clamped).....	10.4 (slow re- traction)
4.42	Both splanchnics stimulated for 1 second.....	11.6
4.45	Both splanchnics stimulated for 0.5 second.....	No effect
4.46	Both splanchnics stimulated for 0.5 second.....	No effect
4.47	Both splanchnics stimulated for 1 second.....	14.0 (slow re- traction)
4.48	Both splanchnics stimulated for 2 seconds.....	13.0 (slow re- traction)
4.50	Both splanchnics stimulated for 2 seconds (with aorta clamped).....	14.8 (slow re- traction)
4.52	Both splanchnics stimulated for 5 seconds.....	11.2 (good re- traction)
4.55	Both splanchnics stimulated for 5 seconds (with aorta clamped).....	11.8
5.00	Clamped aorta for 5 seconds; then stimulated splanchnics for 10 seconds (with aorta clamped)..	11.8
	Aorta released.....	15.4 (further re- traction)

TABLE 8—Continued

TIME		EYE-REACTION AFTER
		<i>seconds</i>
5.07	Clamped aorta for 20 seconds; then stimulated splanchnics for 5 seconds (with aorta* clamped).. Aorta released after 35 seconds.....	No effect No effect
5.09	Splanchnics stimulated for 5 seconds.....	11.2
5.13	Clamped aorta for 10 seconds; then stimulated splanchnics for 5 seconds.....	8.6
5.35	Clamped inferior cava for 35 seconds.....	No effect
5.43	Clamped aorta and cava for 30 seconds.....	No effect
5.44	Stimulated splanchnics for 5 seconds.....	No effect
5.48	Stimulated splanchnics for 10 seconds.....	15.0 (slow)
5.52	Clamped aorta and stimulated splanchnics for 10 seconds.....	15.4
5.56	Clamped cava; then stimulated splanchnics for 10 seconds..... Released cava after 24 seconds.....	No effect 12.4
6.00	Clamped cava; stimulated splanchnics for 10 seconds.. Released cava after 23 seconds.....	No effect 16.6
6.01	0.1 cc. adrenalin (1: 25,000).....	16.6
6.05	0.2 cc. adrenalin (1: 25,000) with aorta clamped...	11.6
6.10	0.1 cc. adrenalin (1: 25,000) with aorta clamped...	9.8
6.12	0.04 cc. adrenalin (1: 25,000)	17.0 (very slow)
6.17 to 6.27	Stimulated splanchnics for 10 seconds in 5 successive observations	No effect
6.30	Massaged lightly, left adrenal.....	No effect
6.31	Massaged strongly, left adrenal.....	No effect
6.32	Massaged lightly, both adrenals.....	No effect
6.33	Massaged vigorously, both adrenals.....	15.0 (slow)
6.35	Stimulated splanchnics for 10 seconds.....	No effect
6.36	0.2 cc. adrenalin (1: 25,000).....	16.8 (good re- traction)
6.40	Heart still beating well, experiment stopped. Left adrenal, weight 0.121 gm. epinephrin 0.07 mg. Right adrenal, weight 0.124 gm. epinephrin 0.07 mg.	

of adrenalin) should still have contained, if anything, more epinephrin than the gland which had only liberated enough to cause the reaction three or four times. It does not seem conceivable that the epinephrin set free could all have come from the

initial store. There is some evidence that the epinephrin set free by massage comes from the store in the adrenals, but that is quite a different thing.

SUMMARY

1. It is shown (on cats and dogs) that the response of the denervated eye to stimulation of the peripheral end of the splanchnic nerves, is due solely to the passage of a substance in the blood stream from the adrenals to the eyeball. For

(a) When the venous path is blocked the response fails, but appears on releasing the block, and at the same interval of time as when the vessels are free. The active substance must therefore have accumulated during the period of stimulation of the nerves behind the block.

(b) When the heart is stopped by stimulation of the peripheral end of the vagus, stimulation of the splanchnics produces no effect on the eye. But on allowing the heart to beat again, the eye response occurs at approximately the same time from the moment of reestablishment of the circulation, as the time interval between stimulation of the splanchnics and the response with the circulation going on normally. During the stoppage of the circulation, by complete cardiac inhibition, accordingly, stimulation of the splanchnics must have caused liberation of the active substance at the same point from which it starts when the splanchnics are stimulated without cardiac inhibition.

(c) When the circulation is slowed without being stopped, as by producing partial inhibition of the heart through the vagus or by hemorrhage, the interval between the beginning of stimulation of the splanchnics and the appearance of the eye response is correspondingly increased.

(d) It is possible to find a strength and duration of stimulation of the splanchnics with which no eye response will be obtained, when the ipsilateral or both carotids are clamped, but which will give a response with the vessels free. With longer or stronger stimulation, a response, but a belated one, may occur even with the carotids clamped. The abolition of the response, and its retardation, can be imitated when appropriate doses of adrenalin

are injected into the femoral vein with the carotids clamped or free.

(e) When adrenalin is injected into the left renal vein, or into the central end of the femoral vein, in suitable amount to produce an eye response approximately equal to that produced by a given stimulation of the splanchnics, the interval of time after which the response follows is sensibly the same for adrenalin injection as for splanchnic stimulation.

2. When the aorta is clamped, and the splanchnic then stimulated, a response may be obtained in the eye while the clamp is still on, or only after its removal, or both during the application and after removal of the clamp. There is some variability in this regard in different experiments. There is also a somewhat greater variability in the time interval at which the response appears, than in observations in which the splanchnics are stimulated with the vessels free, or with the veins clamped. The interpretation of these differences is discussed.

3. Circulation time measurements show that there is always more than sufficient time for a substance to have been carried in the blood from the adrenals to the eye before the appearance of the eye reactions.

4. The latent period of liberation of epinephrin from the adrenals on stimulation of the splanchnics is short since the time interval after which the eye response occurs is sensibly the same whether it is evoked by splanchnic stimulation or by the injection at the level of the adrenals of a quantity of adrenalin sufficient to elicit a response similar in character and amount.

5. The minimum period of stimulation of the splanchnics needed to liberate sufficient epinephrin to elicit a response in the denervated eye is very brief (a fraction of a second). With a current of given intensity the amount of the response increases up to a certain point with the duration of the stimulation.

6. Massage of one or both adrenals causes definite eye response in an animal in which stimulation of the splanchnics has been causing it, and at the same interval of time. When, after repeated excitations of a splanchnic nerve, the reaction on the eye ceases to be obtained, it can still in general be elicited by mas-

sage of the corresponding adrenal. But this reaction is soon exhausted.

7. Good eye reactions have been obtained by stimulation of the splanchnics in cats, in which attempts were made before the experiment to exhaust the epinephrin store of the adrenals, for example, by frightening or by administration of morphin. It did not seem that it was easier to exhaust the capacity of the splanchnic nerves for eliciting these reactions in such animals, than in animals which were guarded as much as possible against preliminary exhaustion of the epinephrin store by psychical disturbances.

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(Note added April 24, 1916.) It has since been shown by two of us (S. & R.) that sufficient epinephrin is liberated from the adrenals in cats (under ether anaesthesia) without electrical stimulation of the splanchnics to give a definite eye reaction. The experiment is done by clamping the inferior cava just above the iliac veins and also clamping the two renal veins, then stripping the cava gently upwards so as to empty it of blood above the clamp and finally clamping it above the adrenal veins. Small branches of the segment of cava have been previously tied. The pocket is allowed to fill with blood from the adrenals. When the clamps are removed the eye reactions are obtained at the same time interval as when the splanchnics are stimulated with the vessels free. After division of the splanchnics (in the thorax) the blood collected in the pocket does not affect the eye unless the splanchnics are stimulated.

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The liberation of epinephrin from the adrenals.

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The solution of the question of the liberation of epinephrin into the adrenal veins and the estimation of the amount so liberated in the absence of artificial stimulation of the splanchnics are complicated by the fact that after withdrawal of blood pressor substances are quickly developed in it, which give the same effect as epinephrin on such objects as the vessels of a frog's legs.² It is therefore desirable to demonstrate the fact of its liberation and to assay its amount without the necessity of withdrawing blood. We have done this (in the cat) by means of the denervated eye reactions (of Meltzer),³ and by the effect on the blood pressure curve.

1. For the eye reactions all that is necessary is to clamp off temporarily a pocket of the inferior vena cava so that only adrenal vein blood enters it. A clamp is applied just above the iliac veins. The renal veins are then clamped and the segment of cava emptied of blood by gently stripping it upwards. Finally a clamp is put on the cava above the adrenal veins. Only a few seconds are occupied in the adjustment of these clamps. Small branches of the segment of cava have been previously tied. The pocket is allowed to fill with blood from the adrenals. When the clamps are removed, the eye reactions are elicited at practically the same time interval as when the splanchnics are stimulated with the vessels free.

¹ *J. of Physiology*, 1911, 43, p. 109.

² Cf. Trendelenburg, *Archiv f. Exper. Path. u. Pharmacol.*, 1915, 79, p. 154.

³ Experiments on the liberation of epinephrin by stimulation of the splanchnics; in which the eye reactions were used, have been described by us elsewhere, *Journal of Pharmacology and Experimental Therapeutics*, 1916, 8, p. 205.

2. After section of both splanchnics (above the diaphragm) the reactions can no longer be obtained. Section of the splanchnics has therefore greatly diminished, if not abolished, the liberation of epinephrin. This is not due to the low blood pressure caused by division of the nerves. For if only the right splanchnic is cut there is little, if any, fall of blood pressure. Nevertheless when the cava pocket is closed off as described, and in addition a clamp is put on the left adrenal vein, the right being free, no eye reaction is elicited on allowing the pocket to empty itself. When the experiment is repeated with the left adrenal vein free the reaction is obtained, although of course less strongly than with both splanchnics intact and both adrenal veins open, since only half the amount of epinephrin is discharged.

3. To demonstrate the effect of epinephrin liberated into a cava pocket upon the blood pressure of the same animal, a somewhat different procedure must be adopted, in order to avoid undue disturbance of the blood pressure curve on forming and on releasing the pocket. The lower end of the cava segment is tied permanently after previous ligation of the abdominal aorta and squeezing of blood from the legs. The renal arteries and veins are also tied. When the eye reactions are available to compare with the blood-pressure curve and manipulation of the intestines is avoided during the application of the upper clamp to the cava segment, it is not always necessary that the circulation through the intestines and liver should be interfered with. Even when the blood pressure curve is somewhat irregular the rise of pressure caused by the liberated epinephrin, occurring at a definite interval after release of the pocket, can be identified by the fact that the eye reaction also commences at or about this moment. However, to further strengthen the evidence we have made experiments in which the celiac and superior mesenteric arteries are first tied off, then the renal arteries, and then the abdominal aorta just below the kidneys. As much blood as possible is got into the anterior end of the animal, and then the inferior cava is tied above the iliacs. The renal veins are then ligated, and the cava pocket now represents only a blind pouch upon the circulation, the filling of which from the adrenal veins, or the emptying of which after removal of the upper clamp produces relatively little mechanical

effect upon the blood pressure. The lower end of the animal is kept raised throughout the experiment. This facilitates emptying of the pocket without manipulation.

4. In different experiments we have assayed, by the injection of known quantities of adrenalin, the amount of epinephrin liberated without artificial stimulation of the splanchnics, under our experimental conditions (narcosis with urethane alone, and with urethane supplemented with ether). For example, in one experiment we found 0.0005 mg. and in another 0.0009 mg. per kg. of animal, per minute. When the pocket is allowed to fill during stimulation of the splanchnics, with intervals of rest, the effect on release is distinctly greater than when it is allowed to fill for the same time without artificial stimulation of the nerves.

5. We have endeavored to measure the amount of blood collected in the pocket, without bringing it into contact with any foreign substance, in the following way: One of the iliac veins is tied near its distal end and the other near the cava. Both iliacs are then divided distal to the ligatures. By means of the ligature on the first iliac it is suspended vertically, while the greater part of the cava segment lies undisturbed. The iliac vein thus serves as the neck of a measuring flask, so to say, the body of which is composed of the cava segment. It is not difficult to determine the moment when the blood, entering the pocket practically without resistance, the walls of the vein being scarcely at all distended so long as the vertical portion of the pocket is empty, just reaches the proximal end of the iliac. If undue exposure of the vein is prevented, a comparison of the flow from the adrenals in successive observations is made possible by noting the intervals of time necessary for the pocket to fill up to this point. The quantity of blood required to fill the pocket can be determined once for all in each animal. The vertical position of a portion of the pocket helps to empty it without manipulation when the clamp is removed.

6. The sensitiveness of the eye-reactions to epinephrin discharged from the adrenals, for example in response to stimulation of the splanchnics, can be increased notably by temporarily clamping off alternative arterial paths. This must be done at such an interval of time after the beginning of stimulation as is not more

than sufficient to allow the epinephrin to reach the beginning of the aorta. A larger proportion of the blood containing the epinephrin is thus forced to take the path to the eye whose reactions are being studied. If, for instance, the left iris is the denervated one, clamping at the proper moment of the thoracic aorta and the innominate markedly increases the reaction. It can be further increased by tying off all accessible branches of the left carotid except those through which the eye must obtain its blood supply.

THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS¹

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It has been stated by various writers that epinephrin is liberated from the adrenals under experimental conditions in the absence of artificial stimulation of the splanchnics and that the liberation is dependent upon the integrity of these nerves. This liberation may be conveniently designated as spontaneous, without implying that it is necessarily a physiological process and not excited merely by the abnormal sensory stimulation, the anesthesia and other factors connected with the experiment.

Tscheboksaroff (1) injected blood collected from the adrenals of dogs after defibrination into small dogs and determined the effect on the blood pressure of blood obtained during stimulation of the major splanchnic with that of blood collected in the absence of stimulation and with the effect of blood obtained after section of both splanchnics. He concluded that section of the splanchnics causes distinct diminution of the adrenalin secretion.

O'Connor (2) using the Laewen perfusion preparation (frog's legs), states that citrate plasma collected from the adrenals in rabbits with the splanchnics intact has a decidedly greater constrictor effect than plasma collected after section of the splanchnics.

The observations of Trendelenburg (3) on the extreme rapidity with which a pressor action is developed in shed blood have shown how difficult it is to be certain that the vasoconstriction

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caused in the Laewen preparation by a given plasma is due to epinephrin. He finds that the pressor action is so quickly developed in blood on being drawn that citrate plasma cannot be used. For in the time required to centrifuge the blood considerable quantities of the vasoconstrictor substances are formed. Even entire citrate blood is already not fresh enough in a fraction of a minute after withdrawal.

In the present position of the question whether epinephrin is normally, or at least under experimental conditions, given off to the blood by the adrenals in the absence of artificial splanchnic stimulation, it seemed desirable to try methods less open to objection, especially so far as the determination of the amount of epinephrin liberated is concerned. As regards the further question whether after section of the splanchnics the discharge is completely abolished or only diminished, we do not see how it is possible to answer it by the aid of methods which permit the development of the pressor substances in the shed blood and depend upon vasoconstrictor reactions of the test objects.

We have endeavored to overcome this difficulty by using a method which does not require withdrawal of the blood to be tested, namely, collection of adrenal vein blood in a pocket of vena cava, which is then released. The presence of epinephrin in the blood is deduced from its action upon the denervated iris or nictitating membrane, and upon the blood pressure of the same animal. The identification of the change in the blood pressure curve produced by epinephrin is greatly assisted by simultaneous observation of the eye reactions. The amount of epinephrin liberated can be estimated by imitating the effect on the blood pressure curve by the injection of appropriate amounts of adrenalin in salt solution.

TECHNIQUE

Cats were employed in the great majority of the experiments. A few dogs were used for special points.

The cava pocket. Where the eye reactions are used alone the cava pocket need not be permanent. For certain purposes the temporary closing off of the pocket for a minute or two at a time is all that is

necessary, and in the interval the circulation proceeds practically in the normal way. A clamp is applied just above the iliac veins. The renal veins are then clamped and the segment of cava emptied of blood by gently stripping it upwards. Finally a clamp is put on the cava above the adrenal veins. Only a few seconds are occupied in the adjustment of these clamps. Small veins entering the cava segment have been previously tied. When the pocket is temporarily clamped off there follows a gradual dilatation of both pupils. On release of the pocket the epinephrin dilatation is easily seen in spite of the initial dilatation. But one advantage of the permanent pocket is that no change occurs in the pupil on clipping off the pocket.

When the blood pressure is studied as well as the eye reactions, the removal of the clamps from the temporary cava pocket generally produces too great a disturbance of the curve. A permanent pocket is therefore formed in the following way:

The coeliac and mesenteric arteries are first tied, then the abdominal aorta at the level of the kidneys. All small veins entering the cava segment are now ligated. Then the renal and lumbar veins are ligated. Meanwhile the blood has been draining out of the intestines and the hind legs. The legs are massaged so as to expel as much of the remaining blood as possible and a ligature is placed upon the inferior cava just above the junction of the iliac veins. The blood pressure is now high, and the animal usually lives a long time. It is kept very warm on a hot table, and the lower end is elevated so as to facilitate emptying of the cava pocket without manipulation. In some experiments the portal vein was tied, in addition, after the blood had well drained from the intestines. The only reason for tying the portal is to make sure that the necessary manipulation in adjusting the clamp on the upper end of the cava segment does not mechanically squeeze so much blood from the liver as to disturb the curve. In the great majority of experiments it was not found necessary or advantageous to tie the portal. The cava pocket thus formed represents only a blind pouch upon the circulation, the filling of which from the adrenal veins or the emptying of which after the removal of the upper clamp produces very little mechanical effect upon the blood pressure. In order to facilitate the application of the clamp a thick soft thread is tied in a loose loop around the cava above the level of the right adrenal. For certain purposes it is advantageous not to tie off the intestinal vessels, and by taking precautions against manipulation of the intestines during formation and release of the pocket, sufficiently

smooth blood pressure curves can be obtained without interrupting the circulation in the digestive tract. It is to be supposed that in investigations concerned with the study of the precursors of epinephrin or of the mutual influence of the adrenals and other abdominal organs, it would be advantageous to form the pocket in this manner. Where the eye reactions are alone being studied there is as already indicated no necessity for crippling any part of the splanchnic area. We have studied in a few animals the eye reactions only, in others only the blood pressure, but in the great majority both eye reactions and blood pressure.

Method of measuring the rate of inflow of blood into the pocket. While it is not necessary to know the quantity of blood entering the pocket in a given time to determine the amount of epinephrin given off in that time it is often desirable to estimate the concentration of the epinephrin in the blood. For this the rate of inflow must be obtained. This can be done, of course, by allowing blood to escape from the pocket as, for example, through a cannula in the left renal vein, and collecting the blood for a given time. Where only one or two observations of the rate of flow are required throughout the experiment this method suffices. To facilitate measuring the flow an indefinite number of times with approximate accuracy and without permitting the blood to escape or to come into contact with any foreign body, we have devised the following method: One of the iliac veins is tied near its distal end and the other near the cava. Both iliacs are then divided distal to the ligatures. By means of the ligature on the first iliac it is suspended vertically while the greater part of the cava segment lies undisturbed. The iliac vein thus serves as the neck of a measuring flask, so to say, the body of which is composed of the cava segment. It is not difficult to determine the moment when the blood entering the pocket practically without resistance, the walls of the vein being scarcely at all distended so long as the vertical portion of the pocket is empty, just reaches the proximal end of the iliac. The more rapid mounting of the blood in the relatively narrow iliac vein is easily seen. As the distal part of the cava segment is itself considerably narrower than the proximal, a fairly sharp reading can also be obtained by suspending the pocket without using the iliac vein. If undue exposure of the vein is prevented, a comparison of the flow from the adrenals in successive observations is made possible by comparing the intervals of time necessary for the pocket to fill up to this point. The quantity of blood required to fill the pocket can be determined once for all

in each animal. The vertical position of a portion of the pocket helps to empty it without manipulation when the clamp is removed.

TABLE 1

Cat 61. Weight, 2.41 kg. Urethane. Cava pocket suspended to estimate time of filling

NO. OF OBSERVATION	TIME	TIME OF FILLING OF POCKET IN SECONDS	BLOOD PRESSURE IN MM.
11-12	3.50	18	42
14		19	38
15		23	40
16		22	40
17		20	38
	4.30	Circulation was now much worse, the heart almost stopped.	
25		101	24
26		108	22
27		125	20

Now filled the pocket to the given level with Ringer's solution. In four successive observations the following quantities were required to fill the pocket: 0.4 cc., 0.35 cc., 0.4 cc., and 0.35 cc.

Left adrenal weighed 0.226 gram, and contained 0.20 mgm. epinephrin.

Right adrenal weighed 0.224 gram, and contained 0.19 mgm. epinephrin.

TABLE 2

Cat 152. Weight, 1.85 kg. Urethane. Pocket suspended as described in technique. Cannula inserted in the left renal vein

NO. OF OBSERVATION	TIME OF FILLING OF POCKET IN SECONDS	BLOOD PRESSURE IN MM.	RISE OF BLOOD PRESSURE
			mm.
4	45	70	8
5	48	66	7
6	45 with asphyxia	64	7-8
7	50 with asphyxia	56	
11	45	50	
12	47	50	
13	55	45	
14	58	45	
15	53	45	

Pocket was now allowed to fill to the given level, tied off and the blood determined by weighing. It amounted to 0.580 gram.

Interpretation of the blood pressure tracings. When eye reactions are available they are of great use in confirming the interpretation of a given rise in the blood pressure curve as an epinephrin rise. Of course where the blood pressure curve remains practically horizontal during the period of closure of the pocket a definite rise in the curve, commencing at a time interval after release of such a magnitude as is known to be associated with epinephrin reactions, can easily be identified as an epinephrin rise without simultaneous eye reactions. It is in the more irregular curves that the eye reactions are particularly valuable. As a rule, it is found that when the blood pressure is relatively low the closing off of the pocket produces little, if any, change in the height of the curve, and therefore the epinephrin rise subsequent to the opening of the pocket starts from a practically horizontal curve (see figs. 5, 6, 14). As already mentioned, even without eye reactions the blood pressure curve then gives perfectly definite proof of the liberation of epinephrin, although with low blood pressure the epinephrin rise is apt to be smaller for a given time of filling of the pocket than with a high blood pressure, since the quantity of blood collected is less. When the blood pressure is high the closing off of the pocket is usually accompanied by a more or less gradual drop of pressure, succeeded on opening the pocket by an immediate and abrupt rise. This initial rise is then followed at the interval appropriate to the epinephrin reaction by another rise (see fig. 10). The difference in the character of the curve associated purely with differences in the initial blood pressure at the time of closure of the pocket are well illustrated in figures 1 and 2. In observation 1, figure 1, with a blood pressure of 30 mm., the curve falls very little during period of closure of the pocket and remains horizontal after opening. In this animal spontaneous liberation of epinephrin was not taking place since one adrenal had been removed and the splanchnic supply of the other cut. In figure 2, observation 5, the initial blood pressure had been increased to 80 mm. by clipping off arteries. On closing the pocket, the curve falls distinctly. When it is opened there is an instantaneous abrupt rise, succeeded by a gradual small rise which brings the curve back to the initial level. No eye reactions whatever were elicited on opening the pocket, and the slight gradual rise does not present the characteristic features of an epinephrin rise.

In the absence of simultaneously elicited eye reactions the question might sometimes be puzzling to a novice in such observations, whether the epinephrin rise was not merely a continuation of the recovery of

the original pressure diminished by the closing off of the pocket. The epinephrin rise, however, by its character and time relations is, when at all considerable, practically always capable of being discriminated with certainty from other elevations which might be present on the curve even when the blood pressure curve shows a good deal of irregu-

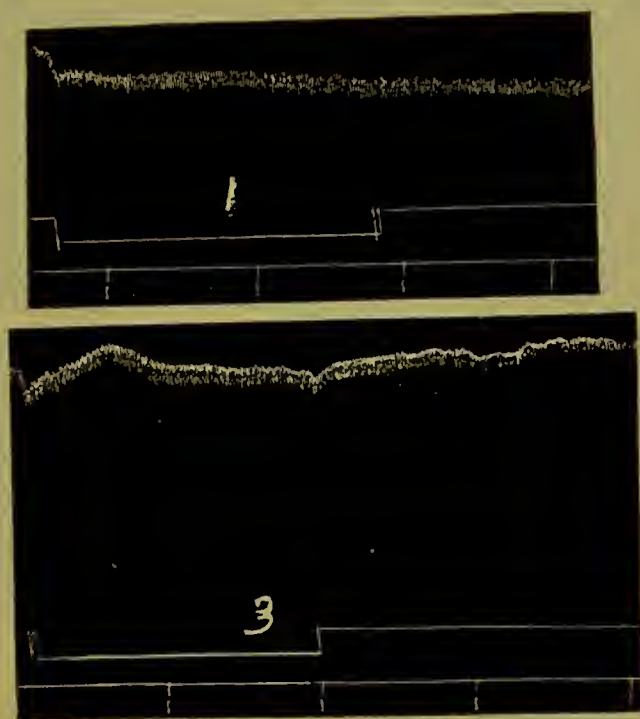


FIG. 1. CAT 81

1, Pocket, showing no liberation of epinephrin by the left adrenal whose splanchnic supply had been cut five weeks previously, and the right adrenal excised. 3, pocket experiment showing the same thing, but with a higher blood pressure, produced by clipping the abdominal aorta. In observation 1, as is generally seen with low blood pressure, the curve is less disturbed by closing and releasing the pocket than with a high blood pressure. In all the figures the time trace gives half minute intervals. The line of zero pressure is the upper signal line unless otherwise indicated.

larity, and in the absence of eye reactions. It is self evident that it is not possible to assay the amount of epinephrin liberated as accurately with an irregular pressure curve as with a regular one. And while extremely slight epinephrin rises can be surely distinguished when eye reactions are also available, a very small rise cannot in the absence of an eye reaction be taken as evidence of liberation of epinephrin,

unless as already stated the blood pressure curve remains practically horizontal during the period of closure of the pocket.

The epinephrin rise is very often preceded by a slight fall of pressure. When this is the case the pupil reaction commences synchronously

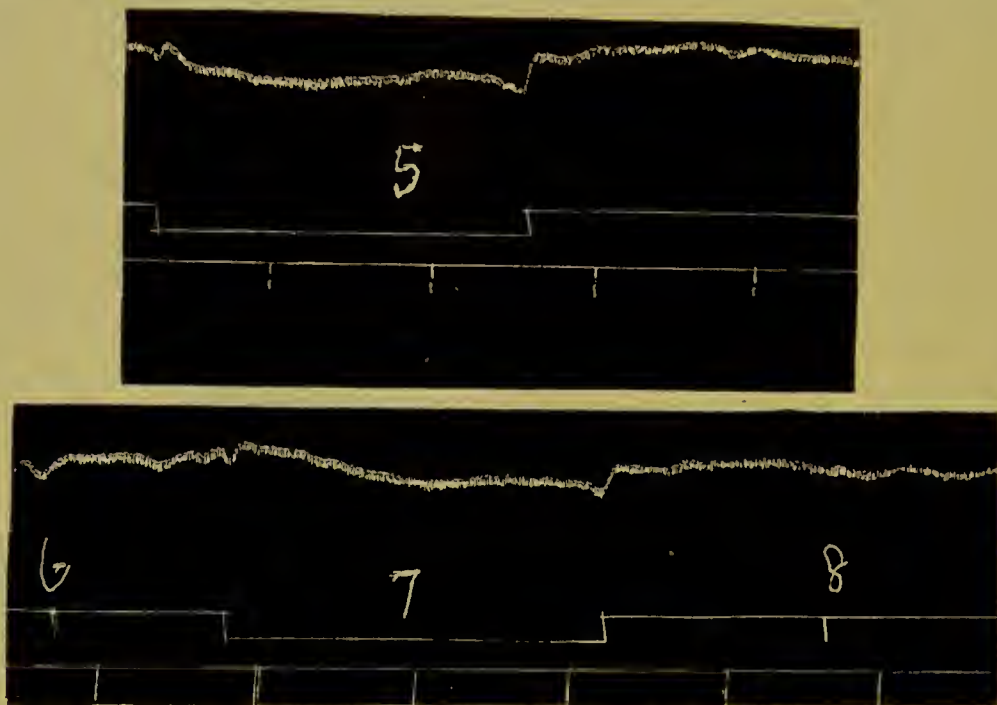


FIG. 2. CAT 81

Animal prepared by excision of right adrenal and section of nerves of left. The tracings illustrate the changes produced in the blood pressure curve by releasing the cava pocket when no epinephrin effect is present. Such changes can be easily discriminated from the effects produced by epinephrin. 5, pocket with left adrenal vein free. Blood pressure was higher than in observation 3, figure 1, and the effect of opening the pocket is correspondingly greater. 7, pocket with left adrenal vein clipped at 6. On releasing the pocket the usual immediate small rise in the blood pressure occurred. It is less pronounced than at 5, as the amount of blood in the pocket was less. Some blood found its way into the pocket in spite of the absence of the right adrenal, possibly through leakage past the lower cava clip. At 8, the clip was removed from the left adrenal and no epinephrin rise occurred. The line of zero pressure has been moved up 25 mm.

with the beginning of the fall (figs. 15 to 18). Elliott (5) states that the curve of blood pressure when the splanchnics are stimulated (the circulation in the splanchnic area not having been interfered with) shows a characteristic drop succeeding the immediate rise, "the cusp of the curve being always placed at the same time interval from the

beginning of the rise. The instant of the turn is that very moment when the nictitating membrane and the other structures of the denervated eye first move. The drop is paradoxically due to the liberation of adrenalin into the blood." In our observations with the cava pocket the same characteristic can often be noted and it undoubtedly affords a criterion of the beginning of the action of the spontaneously liberated epinephrin. When the blood pressure is low this preliminary drop is apt to be less marked (figs. 3, 4 and 14) than with a high blood pressure, or it may be absent (fig. 8). (Compare tables 11 and 12).

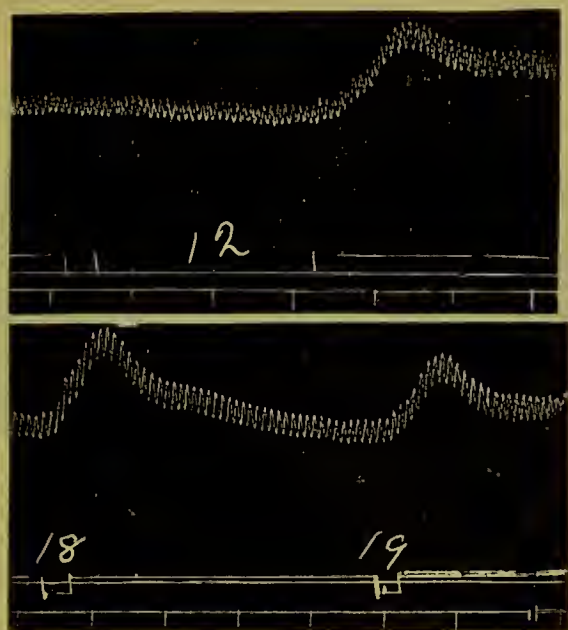


FIG. 3. CAT 116

12, Pocket experiment with stimulation of right splanchnic in abdomen after section of both splanchnics. Stimulation begun at the point indicated on signal line a short time after closure of the pocket. Good rise of pressure after opening the pocket due to epinephrin. 18 and 19, adrenalin injections to assay the amount of epinephrin liberated at 12. 18, 0.3 cc. of adrenalin (1:150,000). 19, 0.2 cc. of the same solution. Reduced to $\frac{2}{3}$.

The method, of course, permits the demonstration that epinephrin is liberated and the assay of its amount not only when the epinephrin is spontaneously discharged but also when it is set free in response to artificial stimulation of the splanchnics. In the latter case the quantity of epinephrin collected in the pocket being greater, the reaction obtained on release is also greater, so that a much more striking de-

monstration of the liberation of epinephrin by splanchnic stimulation is obtainable than in Asher's experiments (4) (see figs. 3 and 4). A smaller output of epinephrin can be detected and assayed in this way, than when the splanchnics are stimulated with the cava open. The experiment can also be made under more physiological conditions than with Asher's technique since, as already stated, it is not absolutely necessary for the pocket experiments to tie off the intestines; and the disturbance of blood pressure curve by the splanchnic stimulation is allowed to disappear before the pocket is opened. Certain other technical details may be mentioned here:

Increasing the eye reaction by clamping alternative arterial paths. The sensitiveness of the eye-reactions to injected adrenalin or to epinephrin discharged from the adrenals, for example, in response to stimu-



FIG. 4. CAT 116

20, pocket experiment with stimulation of right splanchnic in abdomen after section of both splanchnics 21, repetition of 20, but with a shorter time of stimulation. The epinephrin rise after 20 is considerably greater than after 21. $\frac{2}{3}$ reduction.

lation of the splanchnics, can be increased notably by temporarily clamping off alternative arterial paths. This must be done at such an interval of time after the beginning of stimulation as is not more than sufficient to allow the epinephrin to reach the beginning of the aorta. A larger proportion of the blood containing the epinephrin is thus forced to take the path to the eye whose reactions are being studied. If, for instance, the left iris is the denervated one, clamping at the proper moment of the thoracic aorta and the innominate markedly increases the reaction. It can be further increased by tying off all accessible branches of the left carotid except those through which the eye must obtain its blood supply. The best demonstration of the increased effect on the eye of epinephrin discharged in response to splanchnic stimulation is obtained when the discharge is caused by a strong

stimulus lasting only a second or two. All this refers to the cat. In larger animals with a longer circulation time longer periods of stimulation could be employed. For, to intercept the first of the epinephrin, the arteries must be clipped in cats with a good circulation three or four seconds after the beginning of stimulation, and the clipping of the thoracic aorta, of course, interferes with the washing out of epinephrin already liberated into the adrenal capillaries.

It will be seen that except for the very small doses of adrenalin injected there is always a better reaction when alternative paths are clamped. Although there can be no doubt that the total quantity of adrenalin offered to the sensitive structures influences the amount of the reaction in an important degree, it may be assumed that the concentration is also important. Accordingly, below a certain concentration no increase might be expected in the reaction by increasing the amount of adrenalin-containing blood going through the eye.

The quantity of epinephrin which can reach the eye when a reaction is evoked by stimulating a splanchnic nerve, with all the vessels free, is so extraordinarily small that the suggestion is obvious that it might be possible to use the reaction as a test for epinephrin in extraneous liquids in which the concentration can only be very low. To do this it would be necessary to inject as near the eye as possible. A few experiments were made on this point.

Thus, in a dog weighing 13.8 kg., whose left superior cervical ganglion had been excised 24 days previously, injection of an adrenalin solution into the central end of the left superior thyroid artery caused retraction of the nictitating membrane in 5.6 seconds from beginning of injection. The same quantity of adrenalin solution injected into the central end of a femoral vein was followed by retraction of the nictitating in 12.4 seconds. In the cat, results on which are given in table 4, injection of 1 cc. of a 1:150,000 solution of adrenalin into a femoral vein caused moderate dilation of the pupil and retraction of the nictitating in 11.2 seconds. Injection of 0.5 cc. of the same solution by means of a syringe into the central end of the left carotid gave maximal dilatation of the pupil in 2.5 seconds. The heart stopped completely but was re-started by massage. The dilatation of the left pupil was very persistent. Subsequent injection of 1 cc. of 1:700,000 adrenalin into the carotid gave a maximal effect on the pupil in 1 second, while the same amount injected into the femoral vein caused movement of the nictitating in 16 seconds without pupil reaction, the pupil already being dilated to some extent. 0.5 cc. of the same solution

TABLE 3

Condensed protocol of experiment on Cat 19. Weight 2.24 kg. Left superior cervical ganglion exercised 8 days before. Urethane. Adrenalin injected into femoral vein

TIME		ARTERIES CLAMPED AFTER INJECTION	PUPIL DILATATION BEGINS IN
		<i>seconds</i>	<i>seconds</i>
11.48	0.3 cc. (1 : 430,000).....		8.4 moderate
11.49	0.3 cc. (1 : 430,000), aorta clamped..... Repeated with similar result	3	8 moderate +
12.04	0.4 cc. (1 : 850,000).....		10.8 slight
12.05	0.4 cc. (1 : 850,000), aorta clamped..... Repeated with practically same result	3	8.6 moderate
12.10	0.2 cc. (1 : 850,000).....		No reaction
12.11	0.2 cc. (1 : 850,000), aorta clamped.....	4	No reaction
12.23	Left splanchnic stimulated (9 cm. between coils), 4 seconds.....		9.2 moderate -
12.26	Left splanchnic stimulated (9 cm. between coils), 4 seconds, aorta clamped	4	8.2 good +
12.28	Left splanchnic stimulated (9.5 cm. between coils), 4 seconds.....		10 moderate -
12.30	Left splanchnic stimulated (9.5 cm. between coils), 4 seconds, aorta clamped...	4	9 good +
2.48	0.5 cc. (1 : 700,000).....		8 moderate
2.50	0.5 cc. (1 : 700,000) (right subclav., right carotid and aorta clamped).....	4	7.2 good +
2.51	0.3 cc. (1 : 700,000).....		10.2 slight
2.52	0.3 cc. (1 : 700,000) (vessels clamped as at 2.50).....	4	7.4 good +
2.53	0.1 cc. (1 : 700,000).....		No reaction
2.55	0.1 cc. (1 : 700,000) (arteries clamped as at 2.50).....	4	8.8 moderate +
3.15	0.1 cc. (1 : 1,400,000).....		No reaction
3.16	0.1 cc. (1 : 1,400,000) (arteries clamped as at 2.50).....	4	No reaction
3.25	0.5 cc. (1 : 2,900,000).....		No reaction
3.26	0.5 cc. (1 : 2,900,000) (arteries clamped as at 2.50).....	4	12.6 nictitating

Circulation time femoral vein to carotid:

2.0 cc. methylene blue injected, 3.0 seconds

0.5 cc. methylene blue injected, 3.2 seconds

0.5 cc. methylene blue injected, 3.4 seconds

Left adrenal weighed 0.211 gram and contained 0.38 mgm. epinephrin.

Right adrenal weighed 0.189 gram and contained 0.31 mgm. epinephrin.

TABLE 4

Cat 20. Weight 3.2 kg. Left superior cervical ganglion excised 9 days before. Urethane. Adrenalin injected into femoral vein. Both splanchnics cut in thorax at beginning of experiment

TIME		ARTERIES CLAMPED AFTER INJECTION	PUPIL DILATATION BEGINS IN
		<i>seconds</i>	<i>seconds</i>
1.34	1.0 cc. (1: 1,400,000).....		No reaction
1.35	1.0 cc. (1: 1,400,000), aorta clamped	3	No reaction
1.39	0.2 cc. (1: 700,000).....		9 slight
1.40	0.2 cc. (1: 700,000), aorta clamped.....	3	9.2 better
	Repeated last two observations, same result.		
3.00	0.4 cc. (1: 300,000).....		10 moderate
3.01	0.4 cc. (1: 300,000), aorta clamped	3	9 much better
3.40	0.3 cc. (1: 300,000).....		No reaction
3.41	0.3 cc. (1: 300,000), aorta clamped.....	3.5	9 fairly good
	Repeated above observations with same results.		
4.20	0.1 cc. (1: 140,000).....		15 slight
4.22	0.1 cc. (1: 140,000), right subclav., right carotid and aorta clamped.....	3	14.2 very good
4.25	Arteries clamped as at 4.22 but no injection.		No effect
	Repeated observations of 4.20 and 4.22, same results.		
4.35	Tied accessible branches of left carotid		
4.55	0.1 cc. (1: 140,000).....		No reaction
4.56	0.1 cc. (1: 140,000), all arteries clamped as at 4.22.....	3	12.2 good
4.58	0.05 cc. (1: 140,000), arteries clamped as at 4.22.....	3.5	15.2 small reaction
5.00	0.05 cc. (1: 140,000).....		No reaction
5.07	0.2 cc. (1: 140,000).....		12 moderate
5.08	0.2 cc. (1: 140,000), all arteries clamped	3.5	10.8 good
5.20	0.3 cc. (1: 140,000).....		10.2 good
5.21	0.3 cc. (1: 140,000), all arteries clamped	3.5	9.2 very good

Left adrenal weighed 0.169 gram and contained 0.22 mgm. epinephrin.

Right adrenal weighed 0.182 gram and contained 0.22 mgm. epinephrin.

gave good pupil and nictitating reactions in 3.2 seconds when injected into the carotid. 0.5 cc. of a 1: 1,400,000 solution injected into the carotid, and even 0.25 cc. of the same solution, gave fair eye reactions.

The same was true when 0.3 cc. of a 1:3,000,000 solution of adrenalin was injected into the carotid. At this time 1 cc. of a 1:700,000 solution introduced into the femoral vein had no effect on the eye.

Of course, when injection is made into the carotid with a syringe the injection pressure is much more variable than when a burette, raised to a sufficient height, is employed. This accounts for the greater variability in the time intervals of the eye reaction in the experiment just quoted than in that given in table 5.

TABLE 5

Injection of adrenalin into the carotid artery of a cat, by means of a burette

	PUPIL DILATATION BEGINS IN
	seconds
0.4 cc. (1:700,000).....	4.8 good +, also nictitating.
0.2 cc. (1:700,000).....	3.2 good +, also nictitating.
0.3 cc. (1:700,000).....	4.8 good -, nictitating.
0.1 cc. (1:700,000).....	No reaction
0.5 cc. (1:700,000), into femoral vein....	8.8 moderate -

Epinephrin assay. For assaying the amount of epinephrin given off the blood pressure curve is, of course, better than the pupil dilatation. Still by determining the amount of adrenalin just needed to produce a given dilatation of the pupil, very fair results can be obtained with the pupil reaction also. To assay the epinephrin in the blood at a given period of an experiment it is necessary to make adrenalin injections while the conditions are still unchanged. The results of injections of adrenalin made early in an experiment cannot in general be used to estimate the epinephrin given off towards the end of the experiment, since, among other things, the blood pressure is usually higher in the earlier part of the experiment. It is scarcely necessary to add that we always assayed the adrenalin used. For this, and also for the assay of the adrenals, we employed the method of Folin, Cannon, and Dennis, (10) which we compared with the blood pressure method and found to correspond very closely.

The adrenalin solution was injected into the femoral vein when the cava pocket was only temporarily clamped off, into the external jugular when the cava pocket was permanent. It was determined by separate observations that the time interval after which the eye reactions appeared and their amount were sensibly the same whether a given quantity of adrenalin was injected through a cannula into the cen-

tral end of a femoral vein or through a catheter passed up through the femoral vein to the level of the adrenals. At least, this was found to be the case when the liquid ran in through the catheter at the same rate as through the femoral vein. When the orifice of the catheter was in such a position that the solution entered only slowly, the time interval of the eye reactions was, as might be expected, greater than with direct injection into the femoral vein. It was concluded that there was no advantage in point of accuracy in injecting by the catheter rather than into a vein. These observations are illustrated in table 6.

TABLE 6

From an experiment on a 14 kg. dog, narcotized with urethane and ether

	EYE REACTIONS
	<i>seconds</i>
2 cc. (1:35,000), cannula.....	12.8
2 cc. (1:35,000), catheter.....	14.0
2 cc. (1:35,000), cannula.....	11.4
2 cc. (1:35,000), cannula.....	14.2
3 cc. (1:35,000), catheter.....	11.2
3 cc. (1:35,000), cannula.....	11.0
4 cc. (1:35,000), catheter (ran in slowly).....	15.0
4 cc. (1:35,000), cannula.....	11.4
4 cc. (1:35,000), catheter (ran in slowly).....	15.0
4 cc. (1:35,000), cannula.....	13.0
4 cc. (1:35,000), catheter (ran in freely as in vein injection).....	13.0

In one or two experiments we injected the adrenalin solution into the cava pocket through the left renal vein and then released the pocket (fig. 12).

THE SPONTANEOUS LIBERATION OF EPINEPHRIN

For convenience, as already stated, we speak of the epinephrin which is continuously given off under experimental conditions without artificial stimulation of the splanchnic nerves, as spontaneously liberated. In practically all the cats (nearly 40) used by us for these observations we obtained evidence of the presence of epinephrin in the cava pocket blood. We are not, however, able to decide definitely whether this liberation is a normal physiological process merely unveiled by the experiments or an abnormal process dependent on the necessary conditions

of the observations (anesthesia, unavoidable excitation of afferent nerves, etc. (2). The fact that the amount given off per unit of time in cats seems to vary within rather narrow limits even where the experimental conditions, particularly the kind and degree of anesthesia, vary considerably, might perhaps be interpreted as in favor of the first hypothesis. The increase

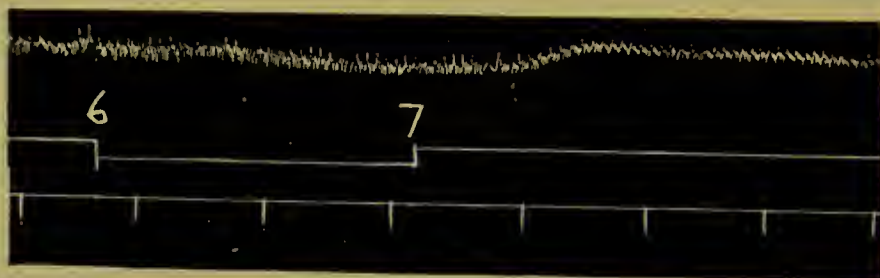


FIG. 5. CAT 57

6 to 7, Pocket experiment with epinephrin rise after release. Blood pressure is low, curve therefore smooth and even a small rise of pressure is capable of identification as an epinephrin rise. The preliminary drop in pressure is absent as is usually the case with low blood pressure. $\frac{1}{4}$ reduction.

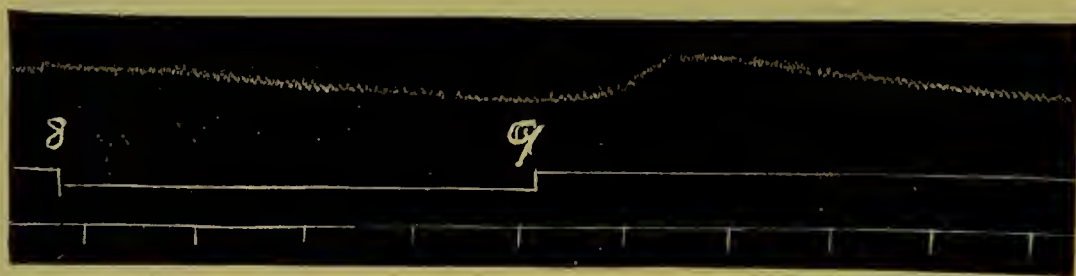


FIG. 6. CAT 57

8 to 9, Pocket. Epinephrin rise on release is greater than that in observations 6 to 7, fig. 5, as the duration of pocket 8 to 9 was greater, and therefore more spontaneously liberated epinephrin was collected. $\frac{3}{10}$ reduction.

in the epinephrin effect upon the blood pressure on release of the pocket with increase in the time for which blood is collected in it is well shown in figures 5 and 6. The liberation is strictly associated with the integrity of the splanchnic nerve supply of the adrenals. At any rate, a discharge which has been perfectly capable of detection and even of assay with the nerves intact, falls at once below the threshold of detection by our methods as soon as the nerves have been divided. It is difficult

to believe that a process governed so definitely by a nervous mechanism has no physiological function. Whether some discharge still goes on after section of the splanchnic supply, could of course only be decided if more delicate methods were available. As already pointed out, positive conclusions based on the vasoconstrictor action of plasmas or blood perfused through frogs' legs cannot be allowed any weight where the quantity of epinephrin which can possibly be present is so small.

The results having been so consistent it is not necessary to multiply protocols. Figures 5 and 6 show that even where the rise of blood pressure is relatively small it may be perfectly definite. The tracings were taken in immediate succession from the same cat, but in figure 6 the period of closure of the pocket was approximately twice as long as in figure 5. It will be seen that the rise of pressure in figure 6 is also approximately twice as great. A condensed record of another experiment follows in table 7. Portions of the tracings of this experiment are given in figure 7.

The protocol of this experiment has been selected for reproduction because the animal had been so prepared that only one adrenal (the left) could liberate epinephrin, the splanchnic supply of the right having been previously cut. The perfectly clear demonstration of the liberation of epinephrin by the left adrenal when its vein was open to the pocket and the complete absence of the reactions when the left adrenal vein was clipped and only the right discharged into the pocket, are striking. As twice as much epinephrin would be given off by the two glands, it is clear that it cannot be a matter of difficulty when both are discharging, to demonstrate the epinephrin reactions. The experiment will be referred to in another connection in the discussion of the question whether after section of the nerve supply the adrenal ever regains the power to liberate epinephrin. Figures 8 and 14 further illustrate the fact that after section of the splanchnic supply of the adrenals the reactions due to the liberation of epinephrin, which were previously present, disappear entirely.

TABLE 7

Condensed protocol of experiment on cat 137; weight 2.71 kg. Al nerve coming to right semilunar ganglion cut 11 days previously. Also the left superior cervical ganglion excised. Urethane, 4 grams by stomach tube. Permanent cava pocket with ligation of coeliae mesenteric and renal arteries and abdominal aorta. Both vagi cut. Cannula in carotid for blood pressure. Cannula in external jugular for adrenalin injection

NO. OF OBSERVA- TION		DURATION OF CLOSURE OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	RISE OF BLOOD PRES- SURE	INITIAL BLOOD PRES- SURE
				mm.	mm.
1	Pocket experiment.....	47	6.8	7	140
2	Clipped left adrenal vein				
3	Pocket experiment.....	45	No	No	
4	Adrenal vein released.....		Good 11.2	6	140
5	Pocket experiment.....	67	Good 6.2	9	145
6	Left adrenal vein clipped.....				
7	Pocket experiment.....	60	No	No	
8	Adrenal vein released.....		Good 9.8	7	
9	Pocket experiment.....	105	Very good 6.2	8	124
10	Ether given.....				
11	Pocket experiment during ether- ization.....	105	Fair 9	*	
12	Ether stopped.....				
13	Pocket experiment.....	105	Very good 9	13	80
15	Pocket experiment.....	105	Very good 7.2	10	96
18	0.3 cc. adrenalin (1:125,000) in- jected.....			18	88
19	0.3 cc. adrenalin (1:250,000) in- jected.....			12	80
20	0.2 cc. adrenalin (1:250,000) in- jected.....			8	82
21	Pocket experiment.....	105	Good 10	8†	
22	Clipped left adrenal vein.....				
23	Pocket experiment.....	130	No	No	
24	Adrenal vein released.....		Small reaction 20	7	

Left adrenal weighed 0.260 gram and contained 0.18 mgm. epinephrin.

Right adrenal weighed 0.286 gram and contained 0.25 mgm. epinephrin.

* The blood pressure curve was spontaneously rising after the drop of pressure due to the ether so that although an epinephrin rise was indicated upon the curve its amount could not be measured.

† The assay of the epinephrin liberated in observation 21 works out at 0.00015 mgm. per minute per kg. of animal for one adrenal. Earlier in the experiment as large a rise of pressure was obtained with a considerably shorter duration of the pocket so that it is probable that when the circulation in the animal was better the liberation of epinephrin per minute was greater than this.

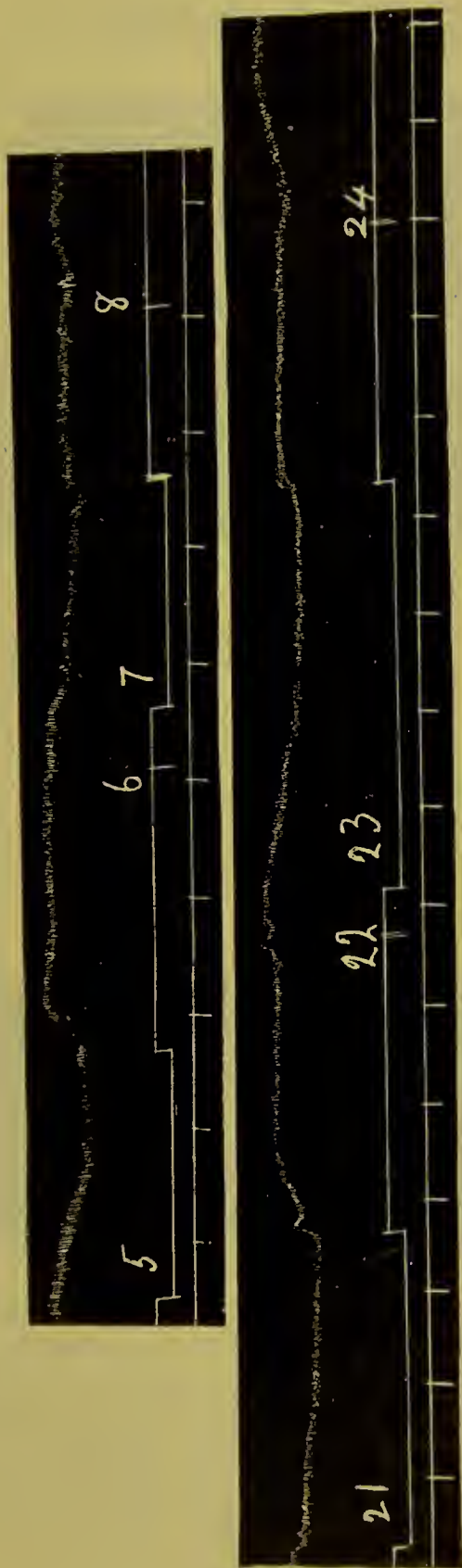


FIG. 7. CAT 137

The tracing illustrates the fact that no spontaneous liberation of epinephrin by the right adrenal after section of its nerves 11 days before could be demonstrated. 5, pocket experiment with both adrenal veins free, definite epinephrin effect with characteristic preliminary small drop of pressure. 7, pocket experiment with left adrenal vein clipped. The clip was put on at 6. No epinephrin effect on release of the pocket. 8, removal of the clip from the left adrenal vein followed by small epinephrin effect. 21, pocket experiment showing liberation (spontaneous) by the left adrenal with a lower blood pressure than at 5. 23, pocket with left adrenal vein clipped at 22. No epinephrin effect on release of the pocket, the pressure merely rising instantaneously to its permanent level and the curve then remaining horizontal till the clip was removed at 24 from the left adrenal vein, when a moderate epinephrin effect occurred. Line of zero pressure has been moved up 55 mm. for the portion of the tracing from 5 to 8 and 25 mm. for the portion from 21 to 24. To get blood pressure add 110 mm. and 50 mm. respectively. $\frac{1}{10}$ reduction.

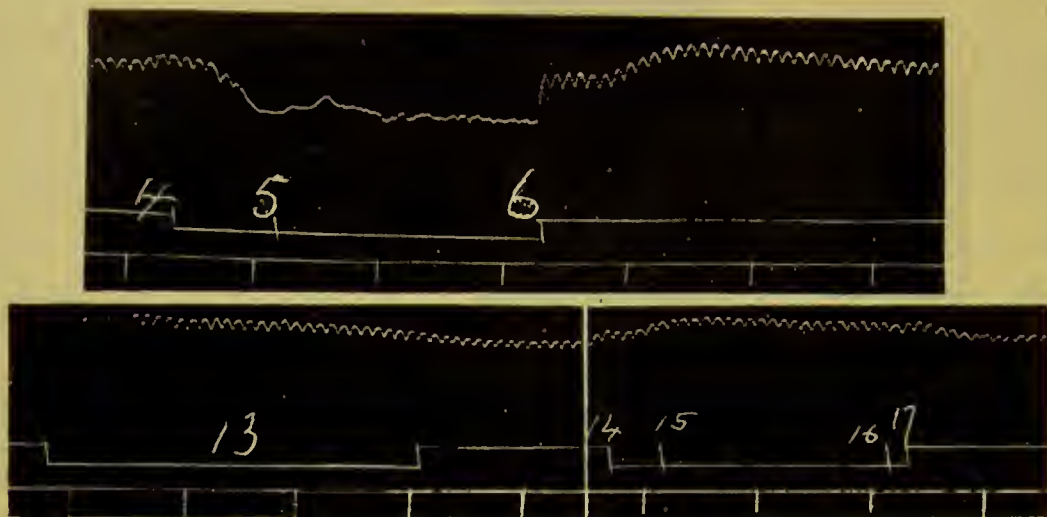


FIG. 8. CAT 117

4 to 6, pocket experiment with stimulation of both sympathetics in the thorax above the diaphragm. Stimulation begun at 5; stopped at 6. Pocket opened at 6. 13, pocket experiment after division of major splanchnics in abdomen and sympathetics in thorax. No epinephrin effect now seen on the blood pressure curve after opening the pocket. 14 to 17, pocket experiment with stimulation of sympathetics in thorax after division of major splanchnics in abdomen. Stimulation began at 15, stopped at 16. No evidence of liberation of epinephrin. $\frac{1}{4}$ reduction.

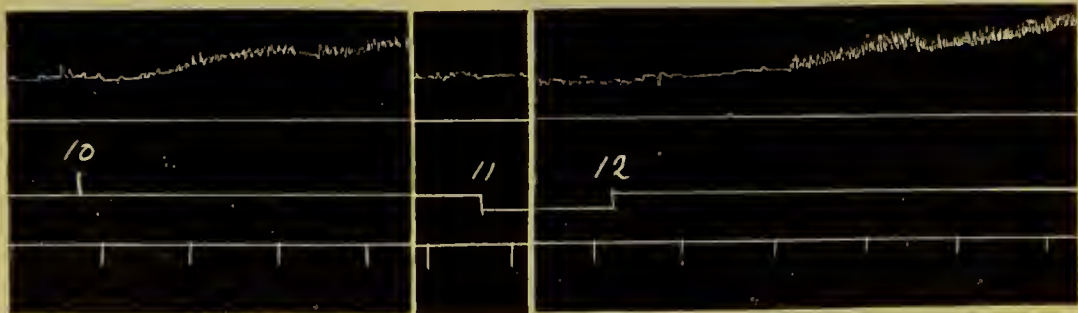


FIG. 9. CAT 57

10, 0.25 cc. adrenalin (1:80,000) injected. The heart had almost stopped but began to beat well again after the adrenalin injection. At 11 to 12, when the heart had again almost stopped and the blood pressure had fallen, a pocket experiment was made. $2\frac{1}{2}$ minutes of the pocket period between 11 and 12 not reproduced to save space. After release of the pocket at 12, the heart was stimulated precisely as by the adrenalin and the blood pressure rose. Line of zero pressure is horizontal line nearest tracing. $\frac{2}{3}$ reduction.

Figure 9 is reproduced because it shows in an interesting way the absolute similarity of the action of injected adrenalin and spontaneously liberated epinephrin. At this stage in the experiment (cat 57) the blood pressure had fallen to an extremely low point and the heart was almost stopped. The injection of 0.003 mgm. of adrenalin at observation 10 caused a marked recovery of the heart and a rise of blood pressure. In the next observation the pocket was closed for $2\frac{1}{2}$ minutes between 11 and 12. On release of the pocket the heart, which in the interval between 10 and 11 had again almost stopped, began to beat well, and the blood pressure rose just as after the adrenalin injection.

Quantitative results. The quantities of epinephrin spontaneously liberated, as estimated by the injection of adrenalin in a number of experiments, are given in table 8. In different experiments the amount per minute per kilo of animal, varied from 0.0003 to 0.001 mgm. with both adrenals discharging. As it is not certain that the rate of discharge has any simple relation to the body weight, the total amount liberated per minute is also given. It varied from 0.0008 to 0.0028 mgm. In one experiment the discharge from a single adrenal was 0.0005 mgm. per minute or 0.00015 mgm. per kilo of body weight per minute. O'Connor by the frog perfusion method obtained in rabbits results ranging from 0.00014 to 0.0007 mgm. per minute per animal.

The effect of section of the splanchnics is not due to the fall of blood pressure. As in animals in which the circulation through the intestines has not been interrupted section of the splanchnic causes a decided drop in blood pressure, it might be asked whether it is not the interference with the blood flow through the adrenals consequent on this fall of pressure which is responsible for the inability to liberate epinephrin. Although in experiments in which the abdominal aorta, the mesenteric and the coeliac arteries have been tied, section of the splanchnics does not cause any considerable fall of pressure, while still abolishing the liberation of epinephrin, the objection was fully taken account of in a number of experiments in which only the right sympathetic

TABLE 8

NUMBER OF ANI-MAL	NUMBER OF OB-SERVATION	BODY WEIGHT KG.	DURATION OF POCKET IN SE-ONDS	INITIAL BLOOD PRESSURE IN MM.	TOTAL EPINEPHRIN IN MG.	TOTAL PER MINUTE	EPINEPHRIN PER KG. PER MINUTE	BLOOD IN POCKET IN GRAMS	ASSAY BY ADRE-NALIN	CONCENTRATION OF EPINEPHRIN
57	11-12	1.83	180	12	0.003	0.001	0.0006	0.308	0.25 cc. 1:75,000	1:1,000,000
59	9	2.36	90	90	0.0017	0.0011	0.0003	0.306	more than 0.25 cc. 1:150,000	1:1,800,000
81	27	2.65	Massage 120	52	0.0016	0.0008	0.0003*		0.2 cc. 1:125,000	
95	22-25	3.275	105 with asphy- xia	140	0.0048	0.0028	0.0008	1.32	0.6 cc. 1:125,000	1:3,000,000
	26		105	130	0.0048	0.0028	0.0008		0.6 cc. 1:125,000	1:3,000,000
114	17	2.435	120	120	0.004	0.002	0.0008	1.255	0.5 cc. 1:125,000	1:3,100,000
	18-21		120 with brachia stim.	120	0.004+	0.002+	0.00085		More than 0.5 cc. 1:125,000	
	22		120	120	0.004	0.002	0.0008		0.5 cc. 1:125,000	
	23-26		120 with asphy- xia	118	0.004+	0.002+	0.00085		More than 0.5 cc. 1:125,000	
	29-32		120 with asphy- xia	120	0.0045	0.0022	0.0009		More than 0.5 cc. 1:125,000	1:2,700,000
	34		120	116	0.005	0.0025	0.001		Less than 0.65 cc. 1:125,000	
	35-38		120 with asphy- xia	114	0.005	0.0025	0.001		Less than 0.65 cc. 1:125,000	

* One adrenal only.

TABLE 8—Continued

NUMBER OF ANI- MAL	NUMBER OF OB- SERVATION	BODY WEIGHT KG.	DURATION OF POCKET IN SEC- ONDS	INITIAL BLOOD PRESSURE IN MM.	TOTAL EPINEPHRIN IN MG.	TOTAL PER MINUTE	EPINEPHRIN PER KG. PER MINUTE	BLOOD IN POCKET IN GRAMS	ASSAY BY ADRE- NALIN	CONCENTRATION OF EPINEPHRIN
116	2	2.76	90	52	0.0033	0.0022	0.0008	1.01	0.5 cc. 1: 125,000	1: 3,000,000
	12		90 with stim. of rt. splanchnic	50	0.0021	0.0014	0.0005	†	0.3 cc. 1: 125,000	1: 5,000,000
	13		90 with stim. of rt. splanchnic	50	0.0021	0.0014	0.0005	†	0.3 cc. 1: 125,000	1: 5,000,000
137	21	2.71	105	140	0.0008	0.0005—	0.00015*		0.2 cc. 1: 250,000	

† This represents secretion from the right adrenal alone during stimulation of right splanchnic. Before observations 12 and 13 both major splanchnics were divided.

*One adrenal only.

including the major splanchnic was divided in thorax. In some of these experiments, section of the right splanchnic was made at the time when the pocket observations were being carried out. In others the connections of the right semilunar ganglion including the major splanchnics were severed beforehand and the animal allowed to recover. When the left adrenal vein was clipped the blood collected in the cava pocket gave no evidence of epinephrin. Removal of the clip after the opening of the pocket is usually followed by epinephrin reactions. The rise of blood pressure following removal of the clip from the left adrenal vein, although it may sometimes be as great as with an ordinary pocket experiment made only a little time before or after, is more gradual and the pupil reaction comes after a somewhat longer time interval. (See for example table 7, observation 21, as compared with observations 22 to 24, and figure

7.) The simplest interpretation of this is that the epinephrin accumulated during occlusion of the adrenal vein, instead of passing at once along the cava as the epinephrin-containing blood collected in the pocket does, must be more gradually washed out of the adrenal vessels. This interpretation is corroborated by the observation that when the adrenal vein is clipped and a cava pocket formed before the clip is removed, so that the accumulated epinephrin-containing blood in the adrenal now escapes into the pocket, the rise of blood pressure and eye reactions elicited on releasing the pocket occur at the same time interval, and have the same character as when the blood is collected in the pocket with the adrenal vein free.

The magnitude of the epinephrin effects obtained by releasing the adrenal vein after a period of occlusion is usually less than when the adrenal vein blood is collected in the cava pocket for the same length of time. This is to be expected. It is, indeed, surprising that the relatively small amount of blood which can be pent up in the adrenal should sometimes contain as much or nearly as much epinephrin as the much larger quantity of adrenal blood which is collected in the same time when the vein is discharging freely into the pocket. An average cava pocket in a cat will contain about 1 gram of blood, or more than double the combined weight of the two adrenals, so that the amount of blood in an adrenal, even when passively congested by clipping its vein, can only be a small fraction of the amount which it discharges into the pocket with the vein free. The concentration of epinephrin must be much greater in the blood behind the adrenal vein clip than in the blood when collected in the pocket. In other words, the amount of epinephrin liberated is not proportional to the quantity of blood flowing through the gland but depends also on the time. If epinephrin is liberated steadily at a fairly constant rate the concentration in the adrenal vein blood must vary inversely with the rate of flow.

Table 9 illustrates the results obtained in one of the acute experiments in which the right sympathetic was divided in the thorax a little above the diaphragm. Portions of the trac-

TABLE 9

Condensed protocol of experiment on cat 37. Weight, 2.45 kg. Left superior cervical ganglion excised 10 days before. Temporary cava pocket method used as described under technique.

NO. OF OBSERVATION	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
					Milli-meters	Begins in seconds	
	9.15	Urethane 4 grams by stomach tube.....					
	10.00	A little ether. Cava pocket prepared. Cannula in carotid and trachea.....					
3	11.35	Pocket experiment....	30	Very good 9.2	22	8	110
4	11.40	Pocket experiment with left adrenal clipped.....	90	Very good 12.8	13	9	100
6	11.43	Pocket with left adrenal vein clipped.....	60	15	5	10	80
7		Removal of adrenal vein clip.....			6		64
	11.50	Thorax opened. Cut right sympathetic above diaphragm....					
	11.55	Clamped abdominal aorta.....					
11	12.00	Pocket experiment with left adrenal vein clipped.....	90	No Slight	No Slight		48
12		Released adrenal vein..					44
14	12.05	Pocket experiment....	75	Very good 22.6	14	20	40
15	12.30	Pocket experiment with left adrenal vein clipped and stimulation of right splanchnic for 2 minutes (5 secs. on and 5 secs. off).....	150	Very good 32	18	20	40
17	12.40	Pocket experiment with left adrenal vein clipped.....	90	No Slight	No		
18		Removed adrenal clip..			10	12	54

TABLE 9—Continued

NO. OF OBSER- VATION	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRES- SURE RISE		INITIAL BLOOD PRESSURE
					Milli- meters	Begins in seconds	
19	12.45	Pocket experiment.....	90	Good 20.2	13	15	58
	2.15	Left symphatetic di- vided in thorax above diaphragm.....					
35	2.20	Pocket experiment with stimulation of right and occasion- ally left sympathet- ics for 3 min., (5 secs. on, 5 secs. off).....	180		14	26	34
38	2.40	Pocket experiment with massage of both adrenals.....	120		18	45	29

Left adrenal weighed 0.197 gram and contained 0.14 mg. epinephrin.

Right adrenal weighed 0.201 gm. and contained 0.16 mg. epinephrin.

ings from this experiment are reproduced in figures 10, 11 and 12. Results in animals in which the splanchnic supply of the right adrenal was divided in advance of the acute experiment have already been quoted in table 7 (fig. 7). They show clearly that the right adrenal no longer discharges a detectable amount of epinephrin. For when the left adrenal vein is clipped, blood collected in the cava pocket produces no reaction (fig. 10), whereas with the left adrenal vein free a good reaction is obtained. When the nerve supply of both adrenals has been cut, pocket experiments are negative, although epinephrin in good amount is liberated by stimulation of the splanchnics (fig. 11) and by massage of the glands (fig. 12)

Does the denervated adrenal eventually regain the power of liberating epinephrin? It is known that cats survive indefinitely when one adrenal is removed and the splanchnic supply of the other cut. If epinephrin has a physiological function, or at any rate an indispensable one, it must be supposed that eventually, even in the absence of innervation, it will be given off from the denervated glands. Elliott (5) speaks of this as something self-



FIG. 10. CAT 37.

14, Pocket experiment showing spontaneous liberation of epinephrin by the left adrenal, the right being eliminated by previous section of the right splanchnic in the thorax. 17, pocket experiment with left adrenal vein clipped. No epinephrin effect on release, till the clip was removed at 18 from the left adrenal vein, when after the usual interval a distinct epinephrin rise occurred due to the epinephrin pent up in the adrenal by the clip. 19, pocket experiment, with left adrenal vein open. Good epinephrin rise. $\frac{3}{10}$ reduction.

evident. "Ultimately," he says, "the glands must be capable of automatic excretion, for the decentralized gland suffices to keep the animal alive." On testing the question, however, we find no evidence that epinephrin in detectable amount is liberated from the adrenals of cats even a considerable time after the innervation has been destroyed.

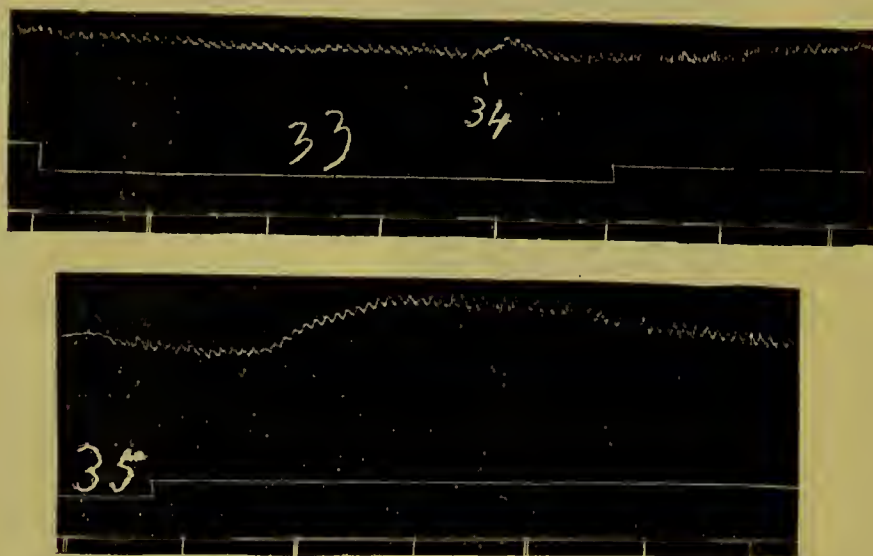


FIG. 11. CAT 37

33, Pocket experiment, after section of both sympathetics in the thorax above the diaphragm. No epinephrin effect on release, the curve rising gradually to the original level and then becoming horizontal just after the end of the portion of the curve we reproduce. 34, elevation of blood pressure curve due to spontaneous movement of the animal. 35, pocket experiment, with stimulation of right sympathetic trunk in thorax. The pocket was closed for three minutes, only the last twenty seconds of which are shown on the tracing. The tracing shows that the right adrenal although liberating no detectable epinephrin spontaneously after section of its nerves is capable of liberating a considerable amount when these nerves are stimulated. Reduced to $\frac{3}{4}$.

The right adrenal was removed from a cat (no. 81) and the fibers coming to the left semilunar ganglion divided. The left superior cervical ganglion had been excised ten days previously. Five weeks after removal of the adrenal, pocket experiments were made, with an absolutely negative result as regards epinephrin reactions on the blood pressure or the eye (fig. 1). Stimulation in the course of the major splanchnic in the abdomen on the left side was also negative. The gland,

however, contained plenty of epinephrin capable of being discharged into its blood vessels, as was shown by massage observations (fig. 13). For example, in observation 27, massage was practiced for two minutes with the cava pocket closed. The rise of blood pressure, accompanied by very good eye reaction 8 seconds after release of the pocket, corresponded to a liberation of 0.0016 mgm. of epinephrin (see table 8).

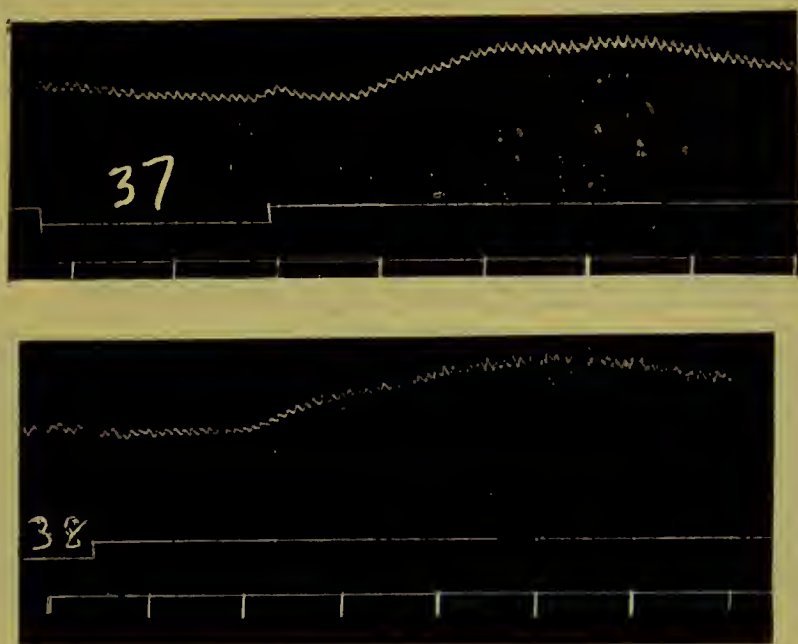


FIG. 12. CAT 37

37, injection into cava pocket of 0.5 cc. of 1:40,000 adrenalin. Pocket released in the usual way in order to compare the effect of the adrenalin with the effect of the epinephrin liberated in pocket experiment 38 by massage of the adrenal. The pocket was closed and massage kept up for two minutes, only the last twenty seconds of which are shown on the tracing. The splanchnic supply of both adrenals had previously been cut. Reduced to $\frac{3}{4}$.

We have shown the same thing in a different way by dividing the nerve supply of the right adrenal, and then, after the animal has recovered, making pocket experiments with the left adrenal vein alternately free and clipped. When the left vein is allowed to discharge into the pocket distinct evidence is obtained of the presence of epinephrin in the blood released, after the clamp is removed from the pocket. But when the left

adrenal vein has been previously clipped, the blood collected in the pocket from the right adrenal produces not the slightest epinephrin effect either on the blood pressure or on the eye. This is not due to the smaller amount of blood collected in a given time. For the negative result is in no wise altered if the period of collection is lengthened. Also, on now sectioning the splanchnic supply of the left adrenal, although the pocket fills as rapidly as before with both adrenal veins free, there are no epinephrin reactions (see tab e 7, fig.7 (cat 137, observations

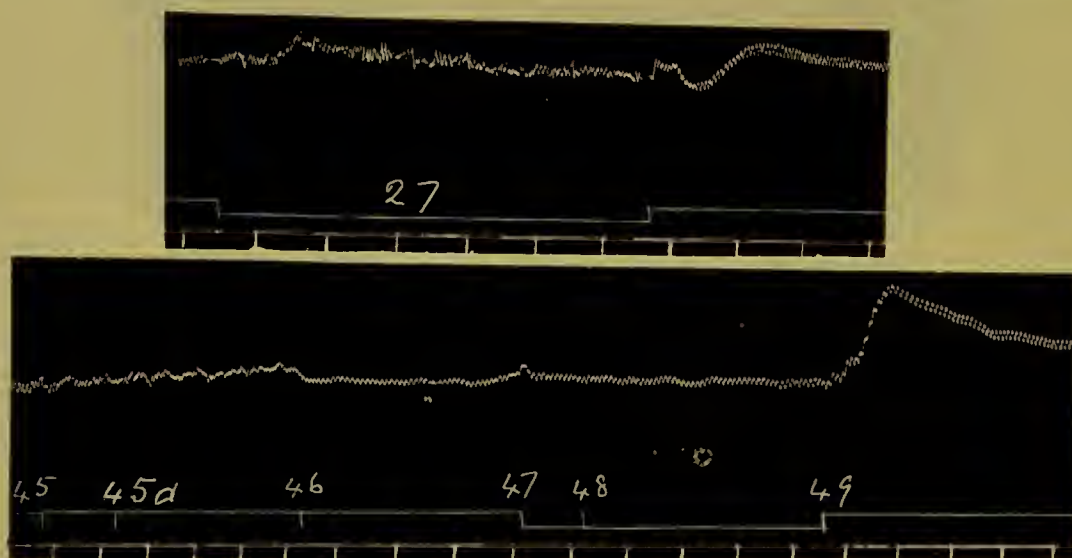


FIG. 13. CAT 81

Showing the effect of massage in liberating epinephrin from the left adrenal whose nerves had been cut 5 weeks before. 27, cava pocket with massage, the left adrenal vein being open to the pocket. 47 to 49, pocket with massage, the left adrenal vein having been closed at 45 and opened at 48, after closure of the pocket. Massage begun at 45-A, stopped at 46. Reduced to $\frac{2}{3}$.

5 to 8 and 21 to 24)). Of course, all that can be deduced from these negative results is that if any epinephrin is spontaneously liberated from the denervated glands, its amount is too small for detection. It was shown that epinephrin could be discharged from the glands under massage in pocket experiments.

Evidence has already been brought forward that considerable amounts of epinephrin can be liberated into the adrenal vessels when the outflow of blood from the gland is prevented. This is the case both for the spontaneous liberation of epinephrin

and for its liberation by splanchnic stimulation. In some of the massage experiments an attempt was made to test the further question whether the epinephrin once liberated into the adrenal vessels can lie there for some time without losing its effect. Massage was practiced in observations on cat 81 with the left adrenal vein clipped. An interval was then allowed to elapse after closure of the cava pocket before the adrenal vein was freed. On opening the pocket good epinephrin reactions were obtained (see fig. 13 (cat 81, observations 45 to 49)) associated with a very good pupil reaction in 9.8 seconds. In another experiment on the same animal the left adrenal vein was clipped, the cava pocket then closed and massage of the left adrenal practiced for two and one-quarter minutes. The pocket was now released the adrenal vein being still clipped. No epinephrin reactions either on blood pressure or eye were obtained. After an interval of $1\frac{1}{2}$ minutes the cava pocket was again closed, the adrenal vein then released and the cava pocket allowed to fill for $2\frac{1}{4}$ minutes without further massage. On release of the pocket excellent eye reactions (pupil and nictitating) and a good rise of blood pressure were obtained.

Either then the epinephrin liberation is not indispensable, or the necessary physiological supply of epinephrin is too small to be detected by methods which clearly detect the epinephrin liberated through the splanchnic nerves under experimental conditions, and also the epinephrin liberated by massage of adrenal glands long deprived of their innervation.

Nature of the spontaneous epinephrin liberation. If good evidence could be adduced that epinephrin is necessary for survival and for health, the question raised on a previous page as to the nature of the epinephrin liberation seen under experimental conditions would seem to be answered by the observations just described—and in this sense, that it is not a discharge of the same order of magnitude as the physiological liberation but a greatly accelerated discharge connected with stimulation of the nervous mechanism controlling the secretion by the abnormal irritation of sensory nerves, or by some action of the anesthetic. If, however, the discharge of epinephrin is essentially

an "emergency function," as suggested by Cannon, it may be that health can be maintained without liberation of epinephrin or with too small a liberation to be detected, although the animal may be handicapped in circumstances which normally evoke the emergency secretion. The increased discharge under experimental conditions might itself be considered an emergency secretion. However this may be, it was seen that cats, with one adrenal removed and the nerves of the other cut, behaved in the same way as cats with intact adrenals, in regard to certain signs of emotional disturbance supposed by some writers to be associated with epinephrin liberation. When the animal was frightened or rendered angry the pupil of the denervated eye dilated well, and dilatation began practically at once, that is to say, much sooner than the same reaction when it is known to be elicited by epinephrin. The dilatation was sometimes as great as, or even greater than the normal eye. In one cat it was constantly greater. The relative amount of dilatation of the pupil in the denervated eye as compared with its fellow was at least as great in all the animals tested as in cats with intact adrenals. The pilomotor effects were not less than in normal cats. In asphyxia and ether anesthesia the pupil of the denervated eye in the cats without adrenal innervation became wider than the pupil of the other eye.

Another point which has some bearing on the question of the nature of the spontaneous liberation of epinephrin seen under experimental conditions, may be mentioned. We have produced evidence in a previous paper (6) that a portion, and usually a very considerable portion, of the epinephrin liberated by electrical stimulation of the splanchnics must be newly formed epinephrin, and cannot have come from the stock in the glands at the beginning of the experiment. The same thing seems to be true of the spontaneous liberation, although, with the nerves intact, there is always a much more definite "spontaneous" loss of epinephrin from the store under experimental conditions than when the cut nerves are stimulated electrically, despite the fact that more epinephrin passes into the blood in a given time with electrical stimulation of the nerves.

Thus, if in cat 37 (protocol, table 9) the discharge from the left adrenal between 11.50 a.m. and 2.20 p.m. was at the smallest rate found in our experiments for one adrenal, namely 0.0005 mgm. per minute, this would amount to 0.075 mgm. for the two and a half hours. The nerves of the right adrenal were cut at 11.50 and those of the left at 2.20. It has been shown by Elliott that section of the nerves protects the store of epinephrin in a gland from discharge. At the end of the experiment the left adrenal contained only 0.02 mgm. of epinephrin less than the right.

In cat 137 the left adrenal lost from its store 0.07 mgm. in 5 hours or over 0.0002 mgm. per minute. The gland discharged spontaneously (see table 8), 0.0005 mgm. per minute.

In cat 116 the major splanchnics were divided after the experiment had proceeded for four and one half hours. The epinephrin liberated before division was estimated at 0.002 mgm. per minute (table 8). If the liberation was at the same rate, say for 4 hours, the amount discharged by the two adrenals would be 0.48 mgm. At the end of the experiment the left adrenal contained 0.11 mgm. and the right 0.09 mgm. The highest content of an adrenal in cats suddenly killed does not exceed 0.35 to 0.38 mgm. Even if the adrenals in this animal at the beginning of the experiment had a maximal load, not less than half the amount discharged must have been new formed.

If the spontaneous discharge is associated with new formation of epinephrin it ranges itself, so far as this fact goes, with the genuine secretions.

Do the major splanchnics carry all the fibers concerned in the liberation of epinephrin? It has been shown by Elliott (5) that section of the major splanchnics alone does not suffice to protect the adrenal (in the cat) from discharge of its epinephrin store. We have found that this is true also for the dog. The question arises whether the major splanchnic carries all the fibers which control the spontaneous liberation of epinephrin, and also, what is probably the same thing, whether it carries all the fibers, artificial stimulation of which causes liberation of epinephrin. We have tried to test this in a number of experiments one of which is illustrated in table 10. Cava pocket observations were made with stimulation of one or both sympathetics in the thorax just above the diaphragm.

The epinephrin reactions obtained on release of the pocket were noted. Then the major splanchnic was divided in the abdomen and stimulation of the sympathetics repeated, with the cava pocket closed for the same or for a longer period. Positive epinephrin reactions on release of the pocket would now, of course, indicate that a portion of the efferent nervous path concerned in the liberation had not been divided. In other observations the spontaneous liberation of epinephrin was first verified by pocket experiments before division of any nerves. The major splanchnics in the abdomen were then cut, and it was noted whether blood collected in the cava pocket still caused any sensible epinephrin reaction. In one experiment (cat 95, see protocol, table 12) we obtained a definite, though small, rise of blood pressure after release of the pocket when the major splanchnics had been previously cut. The rise was associated with a slight pupil reaction. On now dividing the fibers coming to the semilunar ganglion on both sides and repeating the experiment, the result was negative. Before division of the major splanchnics good epinephrin reactions had been obtained. It need not be assumed that after division of such a very important fraction of the total innervation as that carried in the major splanchnic, all cats will show a definite spontaneous liberation of epinephrin. As a matter of fact, we have also seen the opposite result. For instance, in cat 116 (fig. 14, observations 2 to 9) no detectable epinephrin was given off after division of both major splanchnics in the abdomen. Before division of the major splanchnics the amount of epinephrin spontaneously liberated in this animal (at observation 2) was assayed (by means of adrenalin injections such as those shown in the figure in observations 5 and 6) at 0.0008 mgm. per minute per kilo of animal, an amount of the usual magnitude at least. The major splanchnics were then cut, and observation 9 shows no trace of liberated epinephrin. The glands were still at this time perfectly capable of excreting epinephrin. For stimulation of the right splanchnic (fig. 3, observation 12) while the pocket was closed, caused considerable liberation, as shown by the marked rise in blood pressure and eye reactions (pupil, nictitating and

TABLE 10

Condensed protocol of experiment on cat 117. Weight, 1.955 kg. Left superior cervical ganglion excised 26 days previously. Urethane, 4 grams Prepared permanent cava pocket with ligations of arterics. Isolated both sympathetic trunks in thorax above diaphragm without ligating them

NO. OF OBSERVATION		DURATION OF POCKET IN SECONDS	PUPIL DILATA- TION IN SECONDS	BLOOD PRES- SURE RISE		INITIAL BLOOD PRESSURE
				Milli- meters	Begins in seconds	
1	Pocket experiment.....	65	Positive 9	10	7	74
2	Pocket experiment.....	60	Positive 13	8	10	60 +
3	Ligated and cut both sympathetic trunks in thorax.....					
4-6	Pocket experiment with stimulation of both sympathetics 5 secs. on and 5 off, for 90 secs.....	90	Positive 12.4	10*	15	42
8-10	Pocket experiment with stimulation of both sympathetics 5 secs. on and 5 off, for 90 secs.....	90	Positive 11	12	14	42
12	Divided both major splanchnics in abdo- men.....					
13	Pocket experiment.....	100	No	No		34
14-17	Pocket experiment with stimulation of sympathetics in tho- rax for 1 min.....	80	No	No		34
26-29	Pocket experiment with stimulation of right major splanchnic in abdomen for 2 min.....	150	Positive 18.2	3-4*	14	26
30	Pocket experiment.....	120	No	No		20

Left adrenal weighed 0.204 gram and contained 0.17 mgm. epinephrin.

Right adrenal weighed 0.216 gram and contained 0.18 mgm. epinephrin.

Blood pressure has been very low throughout and at the end of the experiment was 30 mm. The pocket was allowed to fill for estimation of the quantity of blood for 3 minutes. It contained 0.45 grams blood.

* This rise although small was perfectly definite.

Notc.—As with the low blood pressures in this experiment a definite preliminary cusp was not seen on the blood pressure curve at the point corresponding to the beginning of the epinephrin effect, the time of commencement of the blood pressure reaction given in the table is always at the beginning of the of rise.

TABLE 11

Condensed protocol of experiment on cat 116. Weight, 2.76 kg. The animal is pregnant. The left superior cervical ganglion was excised 20 days before the experiment. Urethane, 4 grams. 2.00 to 3.20 p.m. pocket prepared. Cannul in carotid connected for blood pressure tracing. Cannula in external jugular vein and in trachea. Coeliac and mesenteric arteries not tied until later

NO. OF OBSERVATION	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
					Milli-meters	Begins in seconds	
2	3.25	Pocket experiment.....	90	Positive 12.6	8	7	42
3		Pocket experiment.....	90	Positive 12.6	8	7	42
4		0.3 cc. adrenalin (1:150,000) injected..			4		42
5		0.6 cc. adrenalin (1:150,000) injected..			10		42
6		0.5 cc. adrenalin (1:150,000) injected..			8		42
7		0.4 cc. adrenalin (1:150,000) injected..			6		42
	4.00	Both major splanchnics divided in abdomen.....					
9		Pocket experiment.....	90	No	No		34
	4.05	Clamped abdominal aorta and coeliac and mesenteric arteries...					
10		Pocket experiment.....	90	No	No		62
11		Pocket experiment.....	90	No	No		70
12		Pocket experiment with stimulation of right splanchnic (off and on).....	90	Very good 13.6	25	9	60
13		Pocket experiment with stimulation of right splanchnic (off and on).....	90	Very good 13.8	25*	11	60
18		0.3 cc. adrenalin (1:150,000) injected..			27		50
19		0.2 cc. adrenalin (1:150,000) injected..			20		50
20	4.55	Pocket experiment with stimulation of right splanchnic (5 secs. on, 5 secs. off).....	150	Very good 12.8	31	10	40

TABLE 11—Continued

NO. OF OBSERVATIONS	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
					Milli-meters	Begins in seconds	
21		P o c k e t experiment with stimulation of right splanchnic (5 secs. on, 5 secs. off)...	90	Positive 16	17	11	42
22		P o c k e t experiment with stimulation of right splanchnic (5 secs. on, 5 secs. off)...	135	Positive 16	12	11	42

Left adrenal weighed 0.185 gm. and contained 0.11 mgm. epinephrin.

Right adrenal weighed 0.200 gram and contained 0.09 mgm. epinephrin.

The smaller rise of pressure in observation 22 as compared with 20 and 21 indicate temporary exhaustion.

The blood pressure at the end was 48 mm. The pocket was allowed to fill for 2 min. The quantity of blood in it was 1.01 gm.

* Curve was practically an exact replica of that obtained in observation 12, and shown in figure 3.

widening of palpebral fissure) on release. The amount of epinephrin liberated during the stimulation was assayed by adrenalin observations, such as 18 and 19, at 0.0005 mgm. per minute per kilo of animal. In comparing this amount with that spontaneously liberated at observation 2, it must be remembered that only one splanchnic was stimulated artificially, and that we have therefore only the output of one adrenal. Further, the splanchnic stimulation did not last for the whole period of closure of the pocket and the nerve was really only stimulated for half the nominal time of excitation (5 seconds stimulation at a time, always succeeded by an interval of 5 seconds rest). These observations, then, form no exception to the general rule that more epinephrin is given off during artificial stimulation of the splanchnic than is spontaneously liberated in the same time. Although this fact would indicate that it might be easier to demonstrate the liberation of epinephrin by artificial stimulation of the sympathetics in the thorax after section of the major splanchnic, than its spontaneous liberation, such experiments

as we have made with stimulation of the sympathetic have not yielded positive results. This may be because of the deterioration of the condition of the animal, indicated by a definite

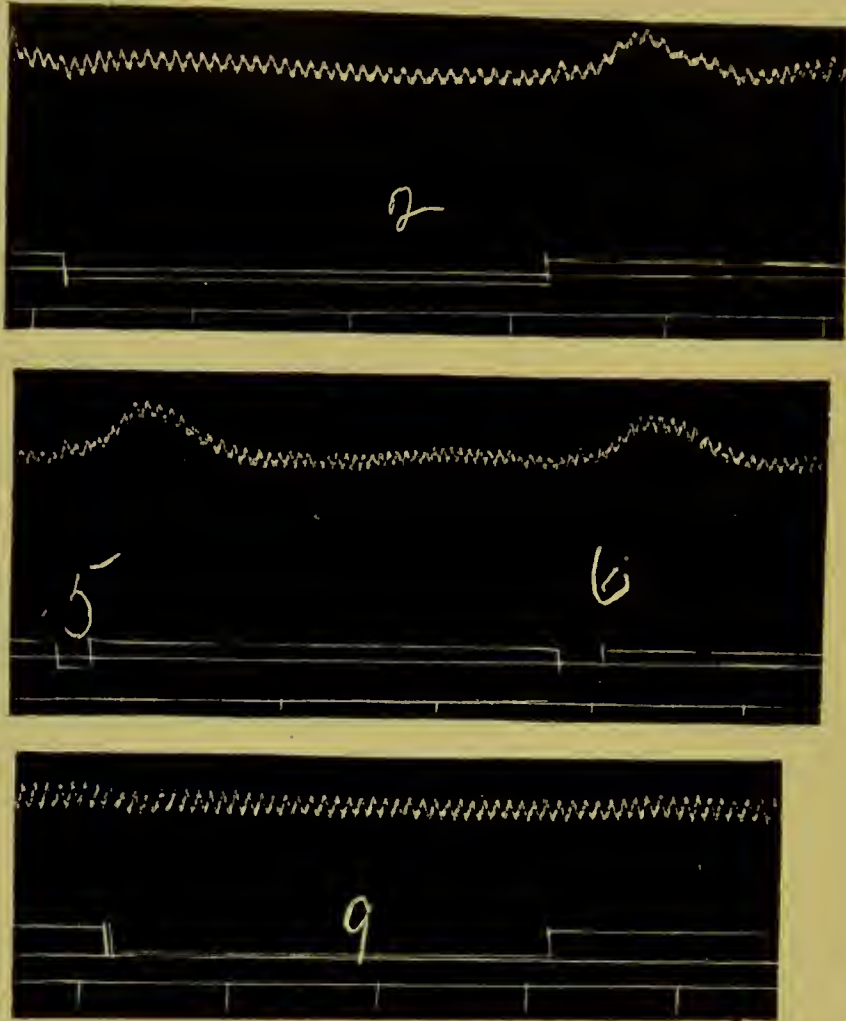


FIG. 14. CAT 116.

2, pocket experiment showing epinephrin rise after releasing the pocket (spontaneous liberation). 5, injection of 0.6 cc. adrenalin (1:150,000). 6, 0.5 cc. of same solution. 9, pocket experiment after section of major splanchnics in abdomen. No evidence of epinephrin liberation. The line of zero pressure is just below upper signal line.

drop in the blood pressure, when, in addition to the rather extensive operation in the abdomen entailed by the making of the cava pocket, the thorax is opened for isolation of the sympathetics. It is, in any case, not at all difficult to understand

that the comparatively slow processes which, in Elliott's experiments on cats and in ours on dogs, are associated with exhaustion of the epinephrin store in an adrenal whose innervation is intact, should produce in 6 or 7 hours very distinct changes, whereas an artificial stimulation lasting for two or three minutes although producing an effect, might not cause a detectable one.

EFFECT OF ASPHYXIA AND STIMULATION OF SENSORY NERVES
UPON THE LIBERATION OF EPINEPHRIN

It has been shown by Elliott (5) that stimulation of sensory nerves causes diminution of the epinephrin store of the adrenals. Cannon and Hoskins (7) have stated that stimulation of sensory nerves and asphyxia produce so great a liberation of epinephrin into the blood that blood collected from the inferior cava by a catheter, passed into it from the femoral vein, gives with intestinal segments marked epinephrin reactions. We have made some experiments by the cava pocket method to test the question of liberation of epinephrin into the blood during electrical stimulation of afferent nerves and during asphyxia. The central end of the brachial nerve was used for stimulation, since with the permanent pocket the sciatic is not available. Asphyxia was produced by closing the tracheal cannula for longer or shorter periods while blood from the adrenals was being collected in the pocket. The asphyxia was stopped some time before the opening of the pocket in order to allow the blood pressure curve, the respiratory variations in which were of course enormously increased during the asphyxia, to become more nearly normal. In some observations the tracheal cannula was not closed until the pocket had been clipped off. In others asphyxia preceded the closing off of the pocket and lasted for a certain time during its closure. The idea was that if asphyxia was producing an increased liberation of epinephrin, and the effect began immediately, the former set of observations would enable blood with a maximum content of epinephrin to be collected, whereas if the asphyxia did not cause its maximum effect until a little time had elapsed, blood richer than normal in epinephrin would

still be caught in the pocket in the latter set of observations. Nevertheless, by neither modification of the experiment have we been able to find any definite increase in the epinephrin liberated during asphyxia as compared with that liberated in control observations in which the animal was breathing normally. If there is any increase at all in such relatively short periods of asphyxia as can be employed (up to about 2 minutes) it is too small to give an unequivocal difference by the methods we have used. With stimulation of the brachial nerves we have not obtained any increase whatever. The experiments are illustrated by a condensed protocol of one of them given in table 12, and by specimens of the tracings reproduced in figures 15 to 18.

It ought in fairness to be stated that the experiments on stimulation of afferent nerves can be done more exactly than those on asphyxia. For first, the blood pressure curve, with the restricted circulation entailed by the making of a permanent cava pocket, is not greatly affected by stimulation of the brachial, and whatever effect is produced ceases practically with the stoppage of stimulation, so that any epinephrin rise after the release of the pocket is easily detected. Secondly, the eye reactions, if present before, are still available after brachial stimulation. On the other hand, asphyxia causes great distortion of the blood pressure curve and also dilatation of both pupils, so that the pupil reaction of the denervated eye cannot be so easily studied. It may further be pointed out that if a slightly greater epinephrin reaction may sometimes appear to be obtained in an asphyxia observation than in the control, the more rapid filling of the pocket with blood due to the increased arterial pressure during the asphyxia might account for the difference.

The experiments on stimulation of the brachial cannot be compared with Elliott's observations on the effect of prolonged afferent stimulation (several hours) in causing exhaustion of the epinephrin store of the adrenals. In any case, there is no reason to suppose that conditions which diminish the stock of epinephrin in the adrenals must necessarily increase the rate of liberation of that substance into the adrenal veins. The diminu-

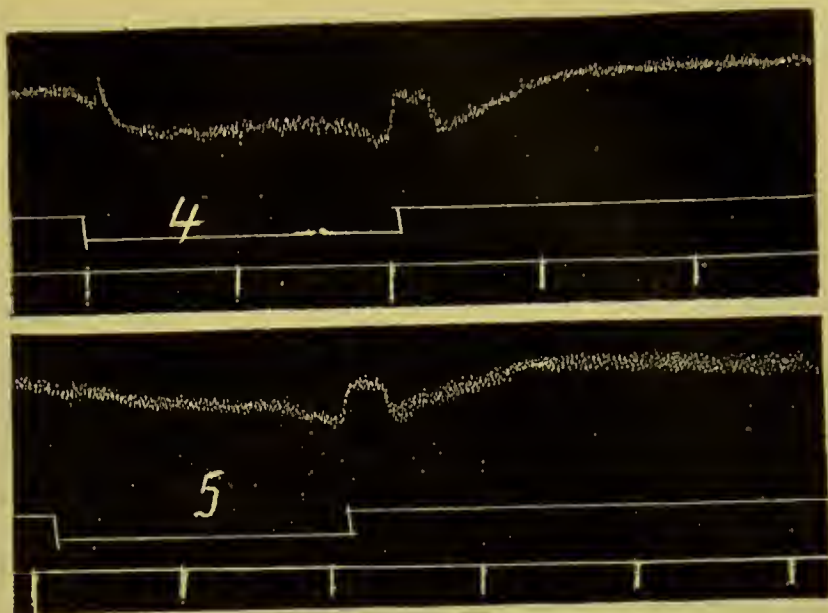


FIG. 15. CAT 95

4, Pocket with brachial stimulation. 5, control pocket without brachial stimulation. Line of zero pressure moved up towards the curve 55 mm.

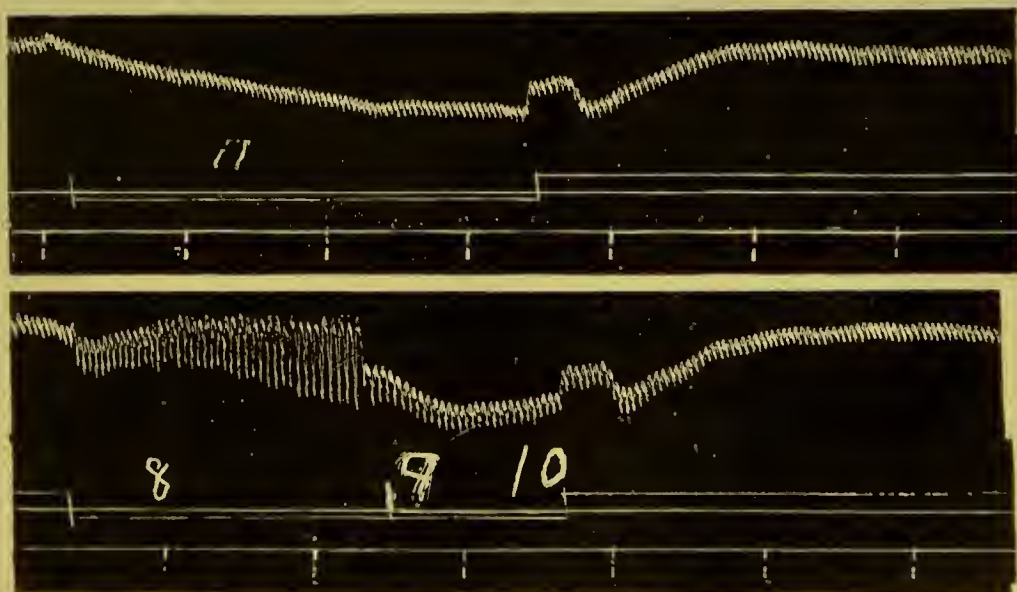


FIG. 16. CAT 95

8, Pocket with asphyxia. Asphyxia stopped at 9, pocket opened at 10. 11, control pocket without asphyxia. Line of zero pressure moved up 50 mm.

tion in the stock may be due to interference with formation of the substance. On the other hand, our observations ought to be capable of comparison with those of Cannon and Hoskins on the liberation of epinephrin into the blood since they also used short periods of stimulation of sensory nerves and of asphyxia. The difference between their results and ours is puzzling. It cannot depend upon the greater sensitiveness of the method

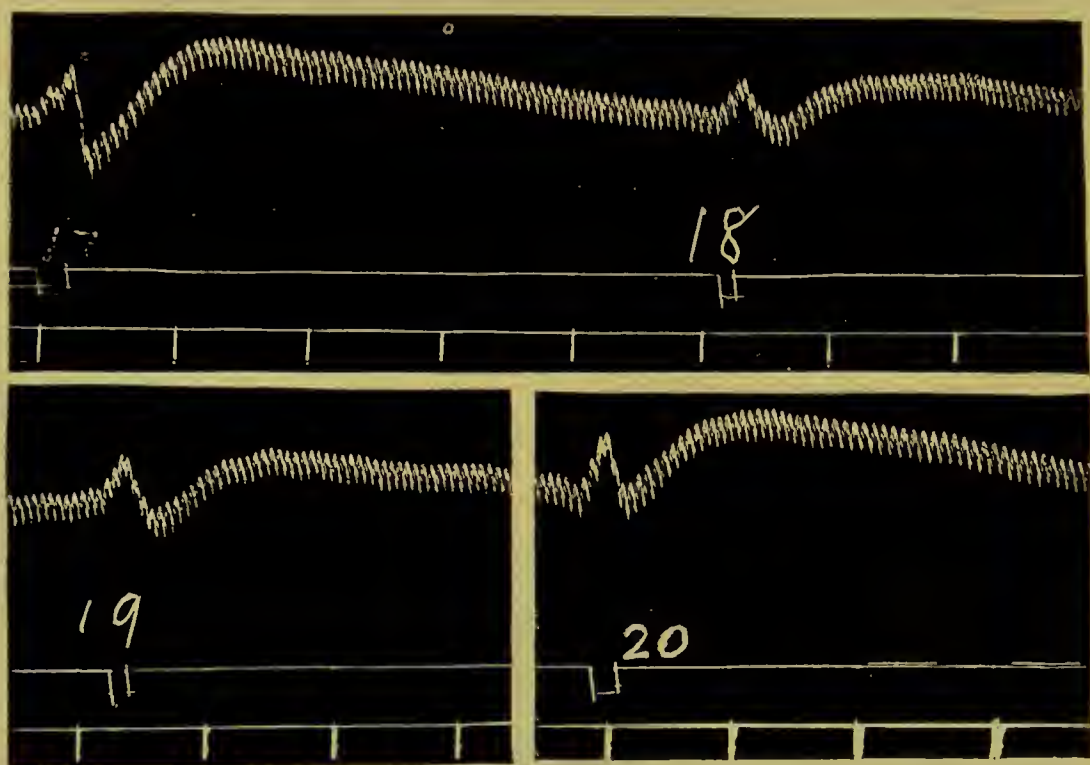


FIG. 17. CAT 95

Adrenalin assay. 17, 0.8 cc. of 1:125,000 adrenalin injected. 18, 0.4 cc.; 19, 0.5 cc.; 20, 0.6 cc. of the same solution. Line of zero pressure moved up 45 mm.

adopted by them (the rabbit's intestine segment method first employed by one of us (S) (8), and by Hoskins (9)). For we obtain positive epinephrin reactions from cava pocket blood collected without asphyxia or electrical stimulation of afferent nerves, whereas Cannon and Hoskins state that cava blood taken by the catheter without asphyxia or sensory stimulation caused no inhibition of the intestinal segments, although blood collected

from the catheter during asphyxia and sensory stimulation caused marked inhibition of the segments. It is, of course, possible that with the more extensive operation in our observations the spontaneous discharge of epinephrin is already so much increased that there is no room for a detectable increase by as-

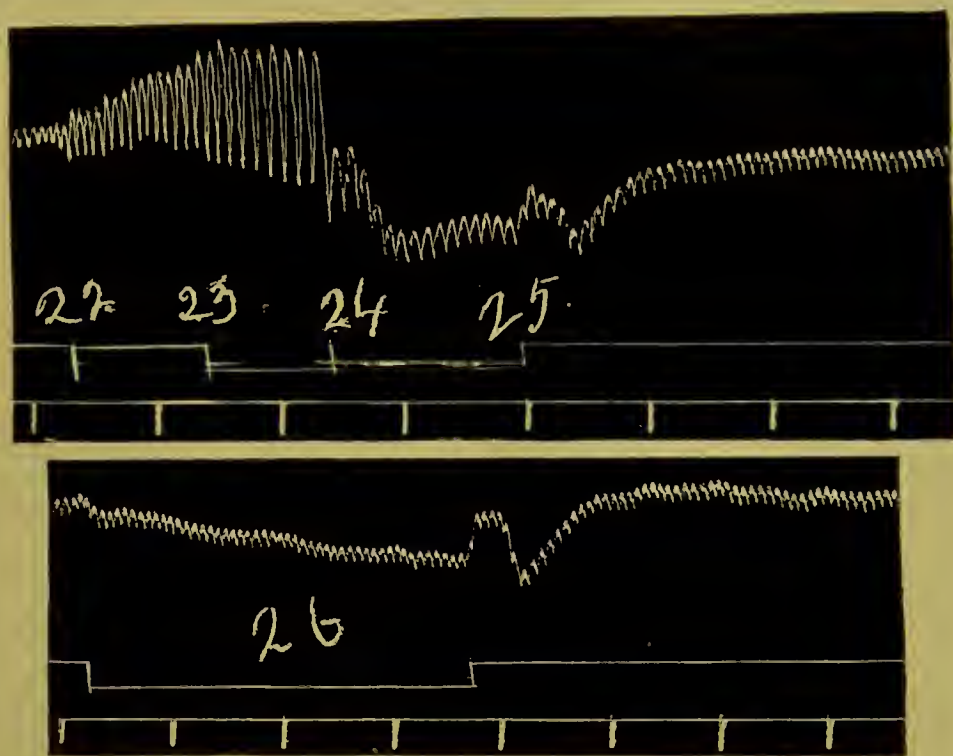


FIG. 18. CAT 95

Asphyxia begun at 22; stopped at 24. Pocket closed at 23, opened at 25 26, control pocket experiment without asphyxia. Line of zero pressure moved up 45 mm.

phyxia, etc. Tschoboksaroff (1) concluded that "the increase of blood pressure caused by stimulation of a sensory nerve (sciatic) has no effect on the quantity of secreted adrenalin." In his experiments also the operative procedure was more severe than in those of Cannon and Hoskins.

TABLE 12

Condensed protocol of experiment on cat 95. Weight, 3.275 kg. Left superior cervical ganglion excised 39 days before the experiment. Urethane, 4 grams. Permanent cava pocket prepared with ligation of arteries. Both vagi divided. Cannula in carotid, external jugular and trachea.

NO. OF OBSERVATION		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
				Milli-meters	Begins in seconds	
1	Pocket experiment.....	50	Positive 7	10	7	150
2	Pocket experiment.....	55	Positive 6	12	6	146
3	Pocket experiment with stimulation of brachial..	62	Positive 7.6	12	7	136
4	Pocket experiment with stimulation of brachial...	64	Positive 6.4	12	6	132
5	Pocket experiment.....	60	Positive 6.6	12	7	138
6	Gave ether.....					
7	Pocket experiment with asphyxia.....	60	Positive 11	*		
8-10	Pocket experiment with asphyxia for 1 minute....	100	Positive 6.2	13	8	140
11	Pocket experiment.....	100	Positive 6.4	11	8	140
17	0.8 cc. adrenalin (1:125,000).....			23		130
18	0.4 cc. adrenalin (1:125,000).....			8		128
19	0.5 cc. adrenalin (1:125,000).....			12		130
20	0.6 cc. adrenalin (1:125,000).....			17		132
22-25	Pocket experiment with asphyxia begun before closing pocket and lasting 1 minute.....	105	Positive 7.2	20	8	140
26	Pocket experiment.....	105	Positive 9.2	20	9	130
	Cut both major splanchnics in abdomen.....					
27	Pocket experiment.....	90	Too wide	6	15	90
28-31	Pocket experiment with asphyxia.....	90	Both dilated.	7	15	100
32-34	Pocket experiment with brachial stimulation....	120	Slight 17.6	9	12	108
38	Pocket experiment.....	100	Slight 14	10	20†	114

* The rise present was disturbed by the spontaneous recovery of the blood pressure from the ether depression so that the amount of the rise could not be determined.

† The beginning of the small fall of pressure which precedes the rise in the epinephrin reaction is what is given in this column in all observations, except 38, in which the cusp was absent.

TABLE 12—Continued

NO. OF OBSERVATION		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
				Milli-meters	Begins in seconds	
39	Pocket experiment..... Both semilunar ganglia freed from strands coming to them	120	Fair 12.6	16	13	114
40	Pocket experiment.....	120	No	No		68
41-44	Pocket experiment with stimulation of brachial	165	No	No		70
	Now allowed pocket to fill for 2 min., 50 secs. Quantity of blood in pocket 1.32 grams.					

Left adrenal weighed 0.200 gram and contained 0.17 mgm. epinephrin.

Right adrenal weighed 0.208 gram and contained 0.18 mgm. epinephrin.

SUMMARY

1. The spontaneous liberation of epinephrin has been studied (in the cat) by means of the (denervated) eye reactions and the blood pressure changes caused by blood from the adrenals when permitted to pass into the circulation from a pocket of the vena cava in which it has been collected in known amounts and for known periods of time.

2. Since the blood is not withdrawn from the vessels the uncertainty introduced by the rapid development in the blood of pressor bodies which simulate the action of epinephrin on some of the objects most generally used in biological tests for that substance, is eliminated.

3. The simultaneous observation of the eye reactions greatly aids in the interpretation of the blood pressure curves when the amount of epinephrin is small.

4. The approximate assay (without withdrawal of blood) of the epinephrin in the blood collected in the cava pocket from the adrenals, by the injection of varying doses of adrenalin generally presents no difficulty. It must be repeated from time to time in the course of an experiment when the condition of

the animal changes. The amount of epinephrin spontaneously liberated in cats was found to vary in different experiments within a rather narrow range considering the differences in the conditions (from 0.0008 to 0.0028 mgm. per minute per animal, or from 0.0003 to 0.001 mgm. per minute per kilo of animal).

5. After section of both sympathetic trunks in the thorax near the diaphragm, including the major splanchnics, the spontaneous liberation of epinephrin is completely abolished. Division of the major splanchnics in the abdomen does not necessarily cause total cessation of the secretion in all cats. In one animal a detectable amount was still liberated but the liberation was entirely stopped when all the fibers coming to the semilunar ganglion were cut.

6. The fall of blood pressure caused by section of both splanchnics has nothing to do with the failure of the adrenals to liberate epinephrin. For when the nerves of the right gland are alone divided and the left adrenal vein clipped, the blood collected from the right adrenal in the cava pocket yields no epinephrin reactions on release of the pocket.

7. Although, as is known, cats survive indefinitely the removal of one adrenal and division of the nerve supply of the other, no detectable epinephrin was found in the blood coming from the remaining adrenal 5 weeks after the operation. Good reactions were obtained on massaging the gland.

8. No increase in the epinephrin liberation was detectable when sensory nerves (brachial) were stimulated. If any increase was produced by asphyxia in our observations it was very slight.

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The alleged exhaustion of the epinephrin store in the adrenal by
emotional disturbance.

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Western Reserve University.]

1. It has been stated that a marked diminution in the store of epinephrin in the adrenal gland is associated with various kinds of emotional excitation. Thus Elliott¹ speaks of morphin-"fright" in cats causing exhaustion of a gland whose splanchnic nerve supply is intact, as compared with the other adrenal whose splanchnic supply has been previously severed. We can confirm his statement as to the difference in the content produced under the influence of morphin but we do not think that fright has anything to do with the result since it is also obtained in dogs where there are no signs of fright.

2. The signs of morphin-"fright" can all be elicited by administering morphin to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no demonstrable liberation of epinephrin through the splanchnics takes place. A cat in this condition behaves identically in the same way as a cat whose adrenal splanchnic supply has been cut on one side but left intact on the other. The pupils are widely dilated and there is the same characteristic restlessness and incessant movement. The content of epinephrin in the remaining adrenal of the first cat is found to be practically the same as that of the adrenal removed before the administration of morphin while the content of the adrenal with intact splanchnic supply in the second cat is definitely diminished.

3. When a cat with the splanchnic supply of one adrenal cut is frightened for many hours by a dog in which also the splanchnic

¹ *Journal of Physiology*, 1912, 44, p. 374.

supply of the adrenal has been divided on one side both animals undoubtedly experience emotions of great intensity. Nevertheless the content of epinephrin in the gland whose nerve supply is intact is not sensibly diminished as compared with the other.

4. We can confirm the statement that β -tetrahydronaphthylamine causes in cats extreme exhaustion of the epinephrin store of an adrenal whose nerve supply is intact as compared with its fellow whose nerve supply has been previously severed.¹ Elliott associates this with the emotional "alarm." We have attempted to test this interpretation by making observations on rabbits.² We have not seen nearly as great a degree of exhaustion in this animal as in the cat. This might be interpreted as in favor of Elliott's view, since signs of "emotional" disturbance are also less marked in the rabbit, although great dilatation of the pupil, increased respiration and other symptoms are present, which, according to Mutch and Pembrey¹ "give the impression that the drug produces a state of increased psychic activity accompanied by muscular action appropriate to the emotions." It seems to us, however, more natural, considering our results with morphin and "frightening" without drugs to interpret the greater effect on the epinephrin content in the cat as due to some other action of the drug than the hypothetical emotional disturbance.

We determined the epinephrin content by the colorimetric method of Folin, Cannon and Denis, which we found to agree sufficiently well with blood pressure observations on the pithed cat.

¹ Elliott, loc. cit.

² Division of the nerves to one adrenal is complicated in the rabbit by the fact that the right adrenal seems to derive a portion of the nerve supply concerned in changes in the epinephrin store from the left splanchnic (Kahn, *Archiv für die gesammte Physiologie*, 1911, CXL, 209; Nishi, *Archiv für Exper. Path. u. Pharmacol.*, 1909, LXI, 401). We therefore tried to eliminate the nervous connections of the left adrenal by dividing all branches going to it from the celiac ganglion and in addition cutting any strands from the lumbar sympathetic and the sympathetic itself below the diaphragm.

THE INFLUENCE OF CERTAIN FACTORS, ESPECIALLY EMOTIONAL DISTURBANCES, ON THE EPINEPH- RIN CONTENT OF THE ADRENALS.*

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In a study, mainly in cats, of the epinephrin discharge caused by electrical stimulation of the splanchnic nerves¹ we were led to consider the question whether the amount of epinephrin liberated by a given strength and duration of stimulation is related to the store of epinephrin already present in the adrenals, or is independent of that store. Desiring to compare the amount liberated in animals whose content was presumably high with the amount liberated in animals whose content was presumably low, we tried to exhaust the store, as far as possible, before the experiment on the liberation was made, by some of the procedures which according to Elliott² cause diminution of the epinephrin load. Morphine was tried and also β -tetrahydronaphthylamine, since Elliott states that these drugs produce marked exhaustion. But as in cats they also cause wide dilatation of the pupil, and we were using the eye reactions as one of the criteria of the presence of epinephrin in the blood, we eventually had recourse to frightening the cat by a dog. Elliott attributes the loss of epinephrin from the glands, induced by morphine and β -tetrahydronaphthylamine, to the fright occasioned in cats by these substances. But he did not make any experiments on the effect of actual fright, although he says that "the most direct

* A preliminary note was published in *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 184.

¹ Stewart, G. N., Rogoff, J. M., and Gibson, F. S., *J. Pharm. and Exp. Therap.*, 1916, viii, 205.

² Elliott, T. R., *J. Physiol.*, 1912, xlv, 374.

method of analysis would be that of inducing emotional fear at once, as by vexing the cat with a dog." He refers to the experiments of Cannon and de la Paz³ as supplying evidence of that nature, since "they showed that the emotion of anger or fear is associated with the appearance of adrenalin in the blood from a cat's suprarenal vein." We do not see that an increased liberation of epinephrin into the blood would necessarily imply a diminution in the epinephrin store of the glands. Elliott himself has given an instance of epinephrin liberation into the blood without any change in the store; namely, the liberation caused by electrical stimulation of the splanchnics. We shall produce evidence in another section of the paper that splanchnic stimulation can induce exhaustion of the epinephrin store, although far less easily than morphine, etc. It is, however, certain that a considerable amount of epinephrin can be discharged into the blood as a result of electrical stimulation of the splanchnics without any appreciable diminution being produced in the store. Indeed, the epinephrin passes into the blood at a more rapid rate than when procedures which distinctly exhaust the store (ether anesthesia, morphine, etc.) are employed. The load of epinephrin present at any given time in an adrenal would seem merely to represent the balance between formation and excretion, its absolute amount giving no index of the rapidity with which the epinephrin is built up and given off. Accordingly, even if it is assumed that fright causes increased liberation of epinephrin into the blood, the question still remains open whether the stock in the glands is diminished.

As a matter of fact, we did not find that in frightened cats the amount of epinephrin which could be excreted into the blood in response to splanchnic stimulation was less than in animals in which the experiments were made in the usual way without preliminary frightening. Nor did the assay of the epinephrin in the adrenals at the end of the experiment indicate that the stock had been appreciably exhausted by the frightening. This induced us to make some observations on the influence of emotional disturbances on the epinephrin content. The content was assayed by the method of Folin,

³ Cannon, W. B., and de la Paz, D., *Am. J. Physiol.*, 1911, xxviii, 64.

Cannon, and Denis.⁴ They compared the colorimetric method with the blood pressure method in pithed cats as described by Elliott and state that the two methods gave the same results. We made comparisons in two experiments and found also a sufficiently close agreement. We used Ringer extracts of adrenals and also acid extracts prepared according to the directions of Folin, Cannon, and Denis.

The colorimetric method proved to be satisfactory for the comparative estimations with which we were alone concerned. Where only small differences in the depth of the tint of the extracts of the two glands were present, we always checked the determinations with the standard by an observation in which the extracts of the two glands were directly compared with each other in the Duboscq colorimeter. The determination was always completed within 3 minutes, at most, after addition of the sodium carbonate solution.

Elliott states that animals killed without section of the nerve supply of one adrenal show equality of load in the two glands. We have seen abundant confirmation of this statement, although occasionally a difference well beyond the limits of error of the epinephrin assay exists, possibly more frequently in dogs than in cats (Table I). A similar equality was found in animals dying spontaneously, either of disease or after operations (Tables XIII and XIV) when the nerves of both glands were intact.

TABLE I.

Species.	Weight of adrenal.		Epinephrin.		Remarks.
	Left.	Right.	Left.	Right.	
	<i>gm.</i>	<i>gm.</i>	<i>mg.</i>	<i>mg.</i>	
Dog. . . .	0.355	0.354	0.51	0.51	Killed with amyl nitrite and ether.
"	1.485	1.720	1.64	1.80	" by bleeding from carotids.
					Thyroid operation 24 hrs. previously.
Cat. . . .	0.238	0.231	0.30	0.26	Killed by chloroform.
"	0.241	0.227	0.30	0.30	Shot through head.

⁴ Folin, O., Cannon, W. B., and Denis, W., *J. Biol. Chem.*, 1912-13, xiii, 477.

TABLE II.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Duration of fright.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.	days	hrs.
Cat 1.....	0.261	0.224	0.28	0.22	5	5
" 2.....	0.326	0.250	0.24	0.25	5	5½
" 3.....	0.225	0.197	0.20	0.16	6	6
" 4.....	0.170	0.176	0.20	0.18	14	5½
" 5.....	0.210	0.221	0.25	0.24	10	5
" 6.....	0.165	0.188	0.14	0.15	33	5
" 7.....	0.154	0.156	0.16	0.18	33	5
" 8.....	0.200	0.196	0.16	0.15	33	5
Dog 1*.....	0.450	0.480	0.67	0.67	8	5
" 2†.....	0.625	0.621	1.00	0.92	5	5

* No. 1 was a small dog.

† No. 2 was a large male dog. Wound infected superficially. Infection did not extend into the abdomen.

Influence of Emotions on the Epinephrin Store of the Adrenals.

In Table II the results of eight experiments on cats and two on dogs are given. In the cats all the fibers coming to the left semi-lunar ganglion, including the major and minor splanchnics, had been divided according to Elliott's method. All operations were, of course, performed under ether anesthesia. Control experiments with morphine and with β -tetra showed that we invariably produced with these drugs the differential action on the two adrenals described by Elliott. Confirmatory evidence of the correctness of our technique was afforded by the marked differential effect observed in a number of the cats which died from various causes (Table XIV). It can, accordingly, be assumed with confidence that the innervation of the left adrenal in the cats used for the experiments was eliminated sufficiently to show definite differential exhaustion of the right, had the emotional disturbance been really associated with exhaustion.

Also, the length of time between the operation and the emotion experiment was sufficient to allow equality of load to be reestablished after the postoperative depletion of the unprotected adrenal, as was shown by control observations. For example, Cat 9 was killed suddenly 4 days after section of the fibers coming

to the left semilunar ganglion. The left adrenal weighed 0.330 gm. and contained 0.37 mg. of epinephrin; the right weighed 0.350 gm. and contained 0.37 mg. Cat 10 was suddenly killed 33 days after section of the fibers coming to the left semilunar ganglion. The left adrenal weighed 0.186 gm. and contained 0.17 mg. of epinephrin; the right weighed 0.200 gm. and contained 0.17 mg. of epinephrin.

The animals were subjected to emotional excitation for 5 to 6 hours by the presence of barking dogs. For this, the cats were enclosed in small cages so constructed that it was impossible for the dog to inflict physical injury upon the cat or to come in contact with it. The stimulation of ordinary sensory nerves was thus excluded as a factor. One of the observers personally took charge of the experiment from beginning to end. Of course, the emotions included fright, anger, fear, etc., the cats showing fight from time to time. The usual signs of sympathetic stimulation, dilatation of the pupil, erection of the hairs of the back and tail, etc., were naturally strongly elicited.

In some experiments dogs previously prepared by interference with the innervation of one adrenal were used to frighten prepared cats, two experiments on emotional disturbance being thus done with the same trouble as one. Control experiments with morphine (Table III) showed that the operations selected permitted a good differential effect, and this was confirmed in the case of prepared dogs dead of infections (pneumonia) (Table XIV).

In Dog 2 (Table II) the left major and minor splanchnics were cut in the abdomen. In Dog 1 the same nerves were divided, but, in addition, nerve strands coming towards the left adrenal from the lumbar sympathetic chain were severed.

It was shown by experiments with morphine that in dogs division of the major and minor splanchnics suffices to give a differential effect. In some of our animals, however, to be certain that enough of the innervation had been eliminated, we excised in addition the two lumbar ganglia next below the diaphragm and cut any strands seen coming from the lumbar sympathetic chain. In two of the animals, three lumbar ganglia were removed, including one above the diaphragm, which was perforated for the purpose. Not all these animals were employed for the observations on emotion; but to save repetition the operations used also for the morphine experiments, to be discussed in the next section, are given here. Some of the morphine experiments are mentioned in this section, since they serve as controls to show that the operations relied on were effective.

It was found that section of the major splanchnic only was not sufficient to give differential protection against exhaustion of the epinephrin store under morphine (Dog 9, Table III). Individual variations, of course, possibly exist in different dogs in this regard. In a dog (No. 17, Table XIV) dead of pneumonia, 13 days after division of the left major splanchnic, the right gland removed after death was found to be exhausted relatively to the left (0.31 mg. of epinephrin as

compared with 0.50 mg.). In another dog (No. 18) also dead of pneumonia, 14 days after section of the left major and minor splanchnics, excision of the two lumbar ganglia immediately below the diaphragm, and section of the strands going to the semilunar ganglion, marked protection of the load of the left adrenal as compared with the right was found (0.30 mg. in the left, 0.08 mg. in the right).

There is no doubt then, that the operations practised by us would have sufficed to reveal a relative exhaustion of the epinephrin store in the gland with the intact nerve supply under the influence of emotions, had such emotions been capable of causing exhaustion.

It will be seen from Table II that neither in the cats nor in the dogs is there any clear and constant deficiency in the epinephrin load of the right (still innervated) adrenal as compared with the left (denervated) gland. A difference of the same order of magnitude as that in Cat 1 may occasionally be seen in cats suddenly killed without operation (Table I). Our experiments yield no evidence that under emotional stress epinephrin is poured out into the blood in such quantities as to produce a decided impression upon the epinephrin store. In observations on the spontaneous liberation of epinephrin under experimental conditions,⁵ we were struck by the steadiness rather than by the mobility of the rate of discharge. Attempts to produce acute changes in the rate in various ways were always without success. We were never fortunate enough to test adrenal vein blood at a moment when an outburst was taking place.

Whether experiments on the epinephrin content of the adrenals under the influence of emotional disturbance continued for a much longer time would yield a different result, we have, of course, no evidence. If the rate at which epinephrin passes into the blood were increased by fright it is conceivable that the rate at which it is built up, accelerated at first to keep the balance even, would eventually decline, thus permitting a deficiency in the load to be established.

Is the Morphine Depletion in Cats Due to Fright?—If emotion *per se* does not cause exhaustion of the epinephrin store the question at once arises whether the morphine effect in cats ought to be attributed to associated fright or to some other action of the drug. An easy way of testing this was afforded by the well known difference

⁵ Stewart, G. N., and Rogoff, J. M., *J. Pharm. and Exp. Therap.*, 1916, viii, 479.

in the action of morphine upon cats and dogs. In dogs no signs of fright are, of course, produced. What happens, then, to the epinephrin load of the unprotected adrenal in dogs, as compared with that of the denervated gland, when morphine is administered? Elliott's result on morphinized cats is easily confirmed, but it is scarcely more difficult to prove that the same qualitative result is obtained on dogs. Accordingly we do not think that fright has anything to do with the morphine effect in cats.

TABLE III.

Morphine Effect on Epinephrin Store in Dogs.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Morphine.	Duration of morphine action.	Nerves cut.
	Left.	Right.	Left.	Right.				
	gm.	gm.	mg.	mg.	days	mg.	hrs.	
3	0.468	0.506	0.83	0.67	3	125*	7	Major and minor splanchnics.
4	0.430	0.384	0.67	0.37	4	125	8½	" " " "
5	0.280	0.284	0.36	0.23	10	150	8	" " " "
6	0.372	0.420	0.42	0.30	15	75	8	" " " "
								and 2 lumbar ganglia and chain excised below diaphragm.
7	0.550	0.565	0.30	0.21	11	80	8	Major and minor splanchnics, 2 ganglia below, and 1 above diaphragm.
8	0.711	0.754	0.97	0.98		150	7½	Control. No operation.
9	0.435	0.466	0.50	0.50	10	150	8	Major splanchnic only.
10	0.346	0.380	0.33	0.27	12	150	8	Only major splanchnic certainly cut.

* Morphine was given in two doses in all the animals, except No. 3, in which it was given in one dose.

Table III illustrates the results obtained with morphine in dogs. Remembering that section of the major splanchnic alone does not protect, or at least not constantly (Dogs 6 and 7), the reader will see that there is uniformly a definite deficiency of epinephrin in the right (innervated) gland as compared with the left (denervated) gland. A control animal (No. 8) morphinized for 7½ hours (without operation) had an equal load in the two adrenals.

TABLE IV.
Control Dogs Suddenly Killed.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Nerves cut.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.		
11	0.640	0.627	1.50	1.20	20 hrs.	Major and minor splanchnics.
12	0.400	0.408	0.72	0.72	9 days.	" " " "
13	0.640	0.550	0.83	0.83	4 "	" " " "

In Table IV results are shown on control dogs, with major and minor splanchnics cut, which were killed without being subjected to the action of morphine. The load in the two adrenals is seen to be equal, except in Dog 11, which was killed 20 hours after the operation. In this animal, as will be shown in the section on the postoperative deficit, the deficiency in the right (unprotected) adrenal no doubt represents merely the deficiency invariably seen after an operation and which has not been recouped in the short interval of 20 hours.

That signs which might be interpreted as those of fright are present in cats under morphine is, of course, not doubtful. Whether this interpretation is correct might be difficult to decide, and does not concern us here. It is, however, of interest to note that epinephrin seems to have nothing to do with those signs.

The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no liberation of epinephrin through the splanchnics takes place. A cat in this condition behaves identically in the same way as a cat whose adrenal splanchnic supply has been cut on one side but left intact on the other. The pupils are widely dilated and there is the same characteristic restlessness and incessant movement. The content of epinephrin in the remaining adrenal of the first cat is found to be practically the same as that of the adrenal removed before the administration of morphine, while the content of the adrenal with intact splanchnic supply in the second cat is definitely diminished.

The dilatation of the pupil of the denervated eye, and the pilotomotor effects associated with fright and anger were also observed

in cats after removal of one adrenal (right) and section of the nerves of the other, when the animals were frightened by a dog and in other ways.

Observations were made on four cats (Table V) in this way.

TABLE V.

Cats with the Right Adrenal Removed and Nerves of the Left Adrenal Cut.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.	days	
11	0.316	0.302	0.25	0.30	1	Died.
12	0.280	0.280	0.19	0.28	9	"
13	0.270	0.250	0.28	0.14	3	Killed by ether.
14	0.220	0.168	0.08*	0.18	37	" after experiments.

* The left adrenal was massaged before removal, in the course of an experiment.

They all yielded the same results. Sudden fright, as by hitting or jerking the holder, caused dilatation of both pupils instantaneously; that is, after an interval too short to be measured with a stop-watch and certainly far shorter than the interval required when reactions are evoked in a denervated eye by epinephrin. Frightening by a dog also caused good dilatation of the pupil on the side on which the superior cervical ganglion had been removed, as well as of the normal pupil. In certain animals the dilatation produced by fright was sometimes even greater than in the normal eye, although in other observations on the same animals it might be less. In other animals the dilatation although quite marked was never so great as in the normal eye.

When the animals were etherized the pupil of the denervated eye in every case dilated more widely than that of the normal eye. The same was true when a moderate degree of asphyxia was produced. In some of the animals repeated observations were made on the effect of fright, asphyxia, and etherization at different periods after the operation up to 5 weeks, always with the same result. In one of the cats (No. 14) an experiment was eventually made to determine whether any detectable amount of epinephrin was being given off in the blood of the adrenal vein, with an entirely negative result.⁵

No difference could be seen in the pupil and pilomotor reactions between these animals and control cats in which one superior cervical ganglion had been excised without interference with the adrenals.

In cats our observations on the effect of morphine upon the epinephrin store of the adrenals agree with Elliott's. For example, in Cat 15 the connections of the left semilunar ganglion were cut and 5 days later 50 mg. of morphine were injected. After 8 hours the cat was killed. The left adrenal weighed 0.212 gm. and contained 0.24 mg. of epinephrin. The right adrenal weighed 0.209 gm. and contained 0.14 mg. of epinephrin.

In interpreting the depletion of the store seen after the long continued action of morphine, ether, urethane, and other anesthetics the possibility must not be lost sight of that the rate of formation or of accumulation of epinephrin in the adrenal may be diminished by the drugs. This would cause depletion of the store if the rate of liberation continued unchanged, just as surely as an increased rate of discharge would cause depletion if the rate of formation remained the same.

Is the Depletion of the Epinephrin Store under the Influence of β -Tetrahydronaphthylamine in Cats Due to Fright?—Elliott has shown that in the cat β -tetrahydronaphthylamine causes marked depletion of the epinephrin store of an adrenal whose nerve supply is intact as compared with its fellow whose nerve supply has been previously severed. We can confirm this statement. For example, in Cat 16, 3 days after section of the nerve supply on the left side 3 cc. of a 2 per cent solution of the drug were injected. After 8 hours the cat was killed. The left adrenal weighed 0.240 gm. and contained 0.22 mg. of epinephrin. The right adrenal weighed 0.200 gm. and contained only a trace of epinephrin.

Elliott associates the exhaustion of the epinephrin store in the cat with the emotional alarm indicated by the behavior of the animal, the wide dilatation of the pupil, etc. Having found reason to doubt the interpretation of the morphine effect on cats as due to emotional disturbance, the obvious suggestion occurred to us to try the effect of β -tetra upon the epinephrin store in the rabbit, an animal in which, according to Mutch and Pembrey,⁶ the symptoms "give the impression

⁶ Mutch, N., and Pembrey, M. S., *J. Physiol.*, 1911, xliii, 109.

that the drug produces a state of increased psychic activity accompanied by muscular action appropriate to the emotions."

It did not prove easy, however, to devise an operation which gave with constancy a good differential effect on the two adrenals. According to Kahn⁷ and to Nishi,⁸ the right adrenal in the rabbit seems to derive from the left splanchnic a portion of the nerve supply concerned in changes in the epinephrin store and in the liberation of epinephrin. Nishi was led to this conclusion by investigations on the glycosuria and hyperglycemia caused by diuretin, and Kahn by the results of his experiments on the relation of the adrenals to puncture glycosuria.

We tried a number of different operations to see whether such a differential effect on the epinephrin store as that described in the section on the postoperative deficit, or as that caused by urethane, ether, and morphine in cats and dogs, or by β -tetra in cats could be produced.

TABLE VI.

Experiments on Rabbits in Which the Right Major and Minor Splanchnics Were Divided.

No. of animal.	Weight of adrenal.		Epinephrin.		Time between operation and death.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.		
1	0.176	0.152	0.16	0.14	14 days.	Killed without drugs.
2	0.160	0.160	0.10	0.10	11 "	" " "
3	0.362	0.300	0.23	0.23	7 hrs.	" " "
4	0.290	0.240	0.13	0.12	14 days.	3 cc. 2 per cent β -tetra, 8 hrs. before being killed.
5	0.250	0.224	0.11	0.10	11 "	5 cc. 2 per cent β -tetra, 7 hrs. before being killed.
6	0.175	0.180	0.10	0.11	14 "	50 mg. morphine, 8 hrs. before being killed.

Table VI shows the results of experiments in which the right major and minor splanchnics were divided in the abdomen. As will be seen, the results were negative. Even in Rabbit 3, which was killed 7 hours after the operation, the content was precisely the same in the two adrenals. In other words, there was no postoperative deficit in the left adrenal after section of the right major and minor

⁷ Kahn, R. H., *Arch. ges. Physiol.*, 1911, cxi, 209.

⁸ Nishi, M., *Arch. exp. Path. u. Pharm.*, 1909, lxi, 401.

TABLE VII.

Experiments on Rabbits in Which the Left Major and Minor Splanchnics Were Divided.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.		
7	0.390*	0.250	0.13	0.14	3 days.	Major and minor splanchnics cut. 50 mg. morphine, 8 hrs. before being killed.
8	0.280	0.270	0.11	0.10	1 day.	Major and minor splanchnics cut, and 1 ganglion near lumbar vein cut out. Died spontaneously.
9	0.400	0.280	0.20	0.13	14 days.	Major and minor splanchnics and strand from lumbar chain cut. 70 mg. morphine in 2 doses, 9 hrs. before being killed.
10	0.276	0.218	0.17	0.18	5 "	Major and minor splanchnics cut. 5 cc. 2 per cent β -tetra, 6 hrs. before being killed.
11	0.309	0.270	0.18	0.16	3 "	Major and minor splanchnics cut. 5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
12	0.296	0.270	0.11	0.11	11 "	Major and minor splanchnics torn from origin through diaphragm and first 2 ganglia below diaphragm excised. 5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
13	0.250	0.216	0.20	0.14	12 "	Major and minor splanchnics, 2 ganglia, and strand from chain cut. 5 cc. β -tetra, 6 hrs. before being killed.
14	0.300	0.300	0.20	0.20	14 "	Major and minor splanchnics and 1 strand cut. 5 cc. β -tetra, 9 hrs. before being killed.
15	0.220	0.180	0.18	0.16	14 "	Major and minor splanchnics and 2 ganglia cut. 5 cc. β -tetra, 8 hrs. before being killed.
16	0.200	0.161	0.16	0.15	3 "	Left semilunar ganglion excised. 5 cc. β -tetra, 8 hrs. before being killed.
17	0.116	0.104	0.11	0.10	$\frac{1}{2}$ hr.†	Major and minor splanchnics cut. Killed as control.

* Edema of left adrenal.

† From beginning of anesthesia till end of operation, 25 minutes. Animal killed $\frac{1}{2}$ hr. after operation. The time is too short for a decided postoperative effect.

splanchnics, the operation affording no protection to the epinephrin store of the right gland as compared with that of the left.

The Epinephrin Store in Postoperative Edema of the Adrenal.—Division of the left major and minor splanchnics was next performed on a series of rabbits. Sometimes, in addition, strands seen coming from the lumbar chain were divided and lumbar ganglia excised. The results are shown in Table VII. They are complicated to a considerable extent by the fact that edema, to which the rabbit's adrenals seem to be susceptible after operations in their vicinity, developed in some of the experiments. This edema is associated with great depletion of the epinephrin store of the affected gland. After a time the edema disappears and the epinephrin reaccumulates. It is obvious that if observations, on the effect of morphine or of β -tetra for example, are made on an animal in which edema of the left adrenal is still present, a genuine diminution in the epinephrin content of the right gland may be completely masked. In spite of this complication, however, Table VII indicates that section of the left major and minor splanchnics does, in some rabbits at least, produce a real differential effect, in contrast to the entirely negative results on section of these nerves on the right side shown in Table VI.

Thus, in Rabbit 9, which received morphine 14 days after the operation the left adrenal contained 0.20 mg. and the right only 0.13 mg. It is practically certain that the other morphine experiment in the table (Rabbit 7) would have shown a similar result but for the edema of the left gland, which was marked. Control observations prove that a gland with this degree of edema, 3 days after operation, never contains nearly so much epinephrin as its fellow. There is, therefore, every reason to believe that before the morphine was given the content of the right adrenal in this animal was considerably higher than that of the left. Since after the morphine period there is practical equality in the two glands, a considerable depletion of the store must be assumed to have taken place. In the dog and cat we have not seen the occurrence of edema of an adrenal in consequence of an operation in its neighborhood. Nor was there any edema in the right adrenal of the rabbit in the experiments in which the right major and minor splanchnic nerves were severed, possibly because the nerves on the right side were divided somewhat farther from the gland than those on the left side and there was accordingly less risk of interference with the lymphatics of the right gland. It is also possible that the mere section of the nerves is a factor in the development of edema, and if the right adrenal in the rabbit derives part of its innervation from the left splanchnics, division of the right splanchnics alone would not be so likely to affect it.

We next tried division of the fibers between the left semilunar ganglion and the adrenal, combined with free separation of the gland from the surrounding connective tissue by passing a blunt dissecting instrument around the greater part of its circumference, in the hope of destroying most of the innervation of the left adrenal while leaving intact such part, if any, of the innervation of the right adrenal as may come from the left splanchnic. As expected, edema of the gland developed, with the concomitant decrease in the epinephrin content already alluded to, only the merest trace being sometimes found. When a sufficient interval was allowed to elapse the edema cleared up and the store of epinephrin was replenished. The results are shown in Table VIII. The marked postoperative deficit seen in Rabbit 22, killed 5 hours after the operation, shows that the left gland was well protected relatively to the right by the operation. Edema of the left had not had time to develop in the few hours which had elapsed. In Rabbit 21, killed half an hour after the operation, no postoperative deficit was shown, the time being too short. This indicates that the marked depletion of the stock of epinephrin through the splanchnic nerves which follows surgical operations develops gradually. A number of the animals were killed as controls to determine the time necessary for recuperation of the epinephrin store.

In 7 days (Rabbit 18) the content of the left adrenal was not yet equal to that of the right. In 4 days (Rabbit 20) the depletion associated with the edema had reached its maximum, only a trace of epinephrin being present in the left adrenal. In 1 day (Rabbit 19), on the other hand, the left adrenal contained if anything, rather more than the right, the postoperative effect on the right (innervated) gland in all probability having not yet entirely disappeared, and the edema effect on the left not having attained its maximum, as is indicated by the relatively small excess of weight of the left gland. In Rabbit 28, which, like Rabbit 20, showed marked edema of the left gland after 4 days, no epinephrin reaction whatever was obtained from that gland.

Such observations as were made with β -tetra and with morphine and other drugs on animals prepared in this way did not yield decisive results for our immediate purpose, as, in the absence of a sufficient number of control animals kept for several weeks, it is not possible to be sure that equality in the epinephrin content of the two glands had been reached before the drugs were administered.

The results, however, seem worthy of being recorded because of the way in which they illustrate the depletion of the epinephrin store

TABLE VIII.

Experiments on Rabbits in Which the Nerves between the Left Semilunar Ganglion and the Gland Were Divided and the Gland Was Separated from the Surrounding Tissue.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.		
18	0.304	0.173	0.22	0.33	7 days.	Killed without drugs.
19	0.170	0.134	0.13	0.11	1 day.	" " "
20	0.440	0.210	Trace.	0.24	4 days.	" " "
21	0.252	0.210	0.24	0.27	$\frac{1}{2}$ hr.*	" " "
22	0.140	0.146	0.11	Trace.	5 hrs.	" " "
23	0.154	0.120	0.12	0.12	15 days.	3 gm. urethane $6\frac{1}{2}$ hrs. before being killed.
24	0.175	0.156	0.11	0.11	17 "	Ether for $2\frac{1}{2}$ hrs. Died of ether.
25	0.390	0.330	0.20	0.15	4 "	5 cc. 2 per cent β -tetra. Died in 6 hrs.
26	0.180	0.122	0.14	0.12	7 "	3 cc. 2 per cent β -tetra, 8 hrs. before being killed.
27	0.190	0.152	0.21	0.21	14 "	3 cc. 2 per cent β -tetra, 8 hrs. before being killed.
28	0.310	0.200	0.00	0.11	4 "	50 mg. morphine, $8\frac{1}{2}$ hrs. before being killed.
29	0.326	0.228	0.14	0.24	7 "	50 mg. morphine, 6 hrs. before being killed.

* From beginning of anesthesia till end of operation was $\frac{1}{2}$ hr. Animal killed $\frac{1}{2}$ hr. after operation.

by conditions leading to edema of the glands after complete or at least extensive section of their secretory nerves and the rate of recuperation of the store of the denervated glands. It is probable that if the animals were kept longer the operation could be used to make tests for differential effects on the two adrenals.

Finally we tried division of the nerves between the semilunar ganglion and the left adrenal, with, in addition, section of any strands seen coming from the lumbar sympathetic towards the adrenal and excision of one or two of the sympathetic ganglia next below the diaphragm. Care was taken to avoid, as far as possible, interference with the gland. This operation gave the best results as regards constancy of effect, although even here the marked differences so easily obtained in dogs and cats were not seen.

TABLE IX.

Experiments on Rabbits, in All of Which the Nerves Were Divided between the Left Semilunar Ganglion and the Adrenal without Disturbance of the Gland. In Some of the Animals Portions of the Lumbar Sympathetic Chain Were Also Cut or Excised.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.		
30	0.324	0.276	0.18	0.14	14 days.	5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
31	0.300	0.260	0.24	0.20	10 "	5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
32	0.188	0.155	0.15	0.13	9 "	2 ganglia nearest diaphragm excised. 5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
33	0.140*	0.103	0.08	0.08	21 "	Strands crossing aorta cut. 5 cc. 2 per cent β -tetra, 8½ hrs. before being killed.
34	0.380	0.400	0.22	0.22	20 "	1 ganglion just below diaphragm excised. 5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
35	0.220	0.182	0.20	0.16	11 "	1 ganglion just below diaphragm excised. 5 cc. 2 per cent β -tetra, 8 hrs. before being killed.
36	0.356	0.314	0.20	0.16	8½ hrs.	Strands crossing aorta cut. Killed as control.

* In Rabbit 33 the left adrenal was found bound down by firm adhesions.

Table IX illustrates the findings. In Rabbit 36, killed to determine whether a postoperative deficit had been established in the right (innervated) gland, a positive result was obtained, although the difference was by no means as large as is often found in rabbits when one adrenal is excised and the other with its innervation intact left in for several hours longer (Table XI).

A number of experiments were made with β -tetra on animals prepared in this way. While in most cases some deficiency in the epinephrin content of the still innervated gland was found after the action of β -tetra, it was never nearly so great as in cats.

In Rabbit 33 the low content (0.08 mg.) in both adrenals is worth noting. The left gland was considerably larger than the right and was still, although 3 weeks

had elapsed since the operation, bound down by adhesions. It is probable, therefore, that the epinephrin store had not yet reached the initial amount. If so, since the right gland also contained only 0.08 mg. after β -tetra had acted for $8\frac{1}{2}$ hours, the drug must have caused some depletion in the unprotected right adrenal. In cats after Elliott's operation on the connections of the semilunar ganglion, we have not seen any edema, and, as he states, equality of the epinephrin content of the two glands is soon established. In one cat (No. 17), however, which was killed as a control, 11 days after the operation on the nerves of the left adrenal, the gland was found firmly bound down by adhesions and the epinephrin content was decidedly less than that of its fellow. The left adrenal weighed 0.398 gm. and contained 0.22 mg. of epinephrin. The right adrenal weighed 0.371 gm. and contained 0.33 mg. of epinephrin.

This is a very exceptional result and was probably due to the fact that we happened to cut a vein in the vicinity, and in securing it some dissection was needed. Such an experiment would, of course, be rejected as a control.

As regards the question raised concerning the way in which β -tetra causes exhaustion of the epinephrin store in cats, it is evident that these observations do not lead us to the same definite conclusion as in the question concerning the mode of action of morphine. The results, indeed, might be interpreted as in favor of Elliott's view that the emotional disturbance in the cat is the efficient factor, since signs of such disturbance are less marked in the rabbit.

It must be kept in mind, however, that the rabbit has a more limited range in the expression of emotion than the cat, and β -tetra certainly produces excitation of sympathetic and other mechanisms which may be concerned in this expression. The pupil is widely dilated, the pulse and especially the respiration are markedly accelerated. There is cutaneous vasoconstriction, the ears being distinctly cool and the ear vessels narrowed. Usually the animals show restlessness and sometimes stamp with the hind feet. The rectal temperature in our observations rose $0.7-1^{\circ}\text{C}$.

The contrast between a rabbit under morphine and a rabbit which has received β -tetra is great. Yet it is easier to demonstrate diminution of the epinephrin store in the rabbit by morphine than by β -tetra. While our observations on morphine seem to indicate clearly that fright is not an essential factor in the morphine depletion, it would be unwarranted to claim that our observations on β -tetra show that fright is not the cause of the epinephrin depletion, so convincingly demonstrated by Elliott in the cat, under the influence of that drug.

It may be permissible to point out, however, that one drug (morphine) which causes different emotional phenomena in the cat and dog produces the same effect on the epinephrin store, while another drug (β -tetra) which causes in the cat and rabbit emotional effects of the same general quality and, perhaps, if the difference in the emotional development of the two animals is taken into account, not so very unequal in degree, affects the epinephrin store very differently.

Comparison of the Epinephrin Load of the Two Adrenals in Rabbits when Removed at Different Times.

Some experiments on rabbits were made in which the left adrenal was first excised, and the right subsequently, after varying periods of time. The results are shown in Table X. It will be seen that in the five animals killed without drugs (Nos. 37, 38, 39, 40, and 41) the right adrenal contains more epinephrin than the left contained at the time of excision. Where the difference is small it could be accounted for

TABLE X.

Experiments on Rabbits to Determine the Load of the Right Adrenal at Varying Intervals after Removal of the Left.

No. of animal.	Weight of adrenal.		Epinephrin.		Interval between excision of 2 adrenals.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.	days	
37	0.454	0.470	0.22	0.28	14	Killed without drugs.
38	0.162	0.190	0.10	0.13	14	" " "
39	0.312	0.324	0.14	0.22	17	" " "
40	0.420	0.346	0.16	0.33	26	" " "
41	0.270	0.176	0.13	0.17	18	Abscess at site of operation, but superficial. Killed without drugs.
42	0.158	0.164	0.16	0.16	16	50 mg. morphine, 7 hrs. before removal of right adrenal.
43	0.450	0.640	0.22	0.47	16	5 cc. 2 per cent β -tetra, 9 hrs. before removal of right adrenal.
44	0.288	0.350	0.18	0.33	16	5 cc. 2 per cent β -tetra, 9 hrs. before removal of right adrenal.

by the diminution of the epinephrin load in both adrenals even during the short operation necessary for removal of the left adrenal. But such a difference as that seen in Rabbit 40, in which the interval between the removal of the two glands was greatest is too large to be explained in this way. In Rabbit 44 this factor was taken into account by removing the second adrenal by an operation which in duration and technique as nearly as possible duplicated the first operation. It must, therefore, be concluded that the load of the remaining adrenal in most of the animals is increased above its amount at the time of excision of the first gland. More observations, especially with longer intervals, would be necessary to make sure that the remaining adrenal can accumulate more epinephrin than is ever seen in one adrenal when both are present. A glance at the other tables which give results on rabbits will show that the number of high loads of the right adrenal in Table X is unusually great for the number of animals, and this is suggestive.

The experiments were originally undertaken with the idea that if the right adrenal regained only its initial content after the operation the animals could be employed for testing the action of substances like morphine or β -tetra on the epinephrin store. The fact that the load increased so much rendered this method too uncertain. The difference in the result in Rabbit 42 which received morphine, and Rabbits 43 and 44 which received β -tetra, is nevertheless striking. The preponderance of the content of the right adrenal in the last two experiments as compared with the left indicates strongly that the influence of β -tetra in discharging the right adrenal was at any rate not great. On the other hand, unless Rabbit 42 was the single exception among the eight animals in the table, some discharge of the right adrenal must have been occasioned by the morphine, since the content after morphine was just the same as that of the left adrenal.

Postoperative Depletion of the Epinephrin Store.

The fact has already been alluded to that during and for some time after operations a more or less progressive decline takes place in the epinephrin store of an adrenal whose nerve supply is intact as compared with its denervated fellow. The phenomenon was well illustrated in experiments in which one adrenal was removed, and then after varying intervals of time the other. The exact opposite of the results shown in Table X is then seen; that is, a marked deficiency in the second adrenal as compared with the first. For example, in

Rabbit 45 (Table XI) the left adrenal was excised and contained 0.37 mg. of epinephrin. The wound was sewed up, the whole operation lasting 20 minutes, and after $8\frac{1}{2}$ hours the right adrenal was removed. It contained only 0.13 mg. of epinephrin.

TABLE XI.

Experiments Showing Postoperative Depletion of Epinephrin Store of One Adrenal as Compared with the Other Removed at Operation.

No. of animal.	Weight of adrenal.		Epinephrin.		Interval between excision of 2 adrenals.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.		
Rabbit 45.....	0.394	0.384	0.37	0.13	$8\frac{1}{2}$ hrs.	
Cat 18.....	0.220	0.218	0.18	0.14	5 "	
" 19.....	0.205	0.238	0.22	0.25	2 days.	
Rabbit 46.....	0.318	0.314	0.20	0.11	$6\frac{1}{2}$ hrs.	5 cc. 2 per cent β -tetra, $6\frac{1}{2}$ hrs. before being killed.
" 47.....	0.092	0.084	0.14	0.08	7 "	50 mg. morphine, $7\frac{1}{2}$ hrs. before being killed.
" 48.....	0.267	0.238	0.11	0.09	$6\frac{1}{2}$ "	50 mg. morphine, $6\frac{1}{2}$ hrs. before being killed.
" 49.....	0.380	0.408	0.28	0.12+	8 "	50 mg. morphine, 8 hrs. before being killed.
Cat 20.....	0.240	0.300	0.33	0.20	6 "	50 mg. morphine, 6 hrs. before being killed.
" 21.....	0.225	0.258	0.22	0.14	7 "	50 mg. morphine, 7 hrs. before being killed.
" 22.....	0.230	0.221	0.26	0.26	8 "	Right adrenal excised and nerves of left adrenal cut. 50 mg. morphine 8 hrs. before being killed.
Dog. 14.....	0.354	0.453	0.67	0.42	8 "	175 mg. morphine 8 hrs. before being killed.
" 15.....	0.352	0.360	0.38	0.30	7 "	200 mg. morphine 7 hrs. before being killed.

This great depletion was assuredly not present at the end of the operation, as shown in other experiments. Etherization for 20 or 30 minutes does not produce a great impression on the store of an adrenal removed at the end of that time. Unless, then, the depletion

is an after-effect of the short etherization, which does not seem likely, it must be due to some other factor, probably the sensory stimulation acting during the postoperative period.

Similar results in animals with one gland protected by section of its nerves have already been given in other tables; for example, Rabbit 22 (Table VIII). 5 hours after section of the nerves of the left adrenal, the animal was killed. The right adrenal contained only a trace of epinephrin, the left 0.11 mg. In the same table and in Table VII examples are given where in half an hour after the operation practically no postoperative difference was present.

The recuperation of the store is usually evident in 1 day, although it may not then be complete. For instance, in Cat 19 (Table XI), 2 days after removal of the left adrenal the right contained 0.25 mg. as compared with 0.22 mg. in the left. Probably the difference represents about the amount by which the store of the left adrenal was diminished during the short operation for its removal. Many of the animals received morphine immediately after excision of the first adrenal (always the left except in Cat 22), and one (Rabbit 46) received β -tetra. The effect, if any, of these drugs is complicated by the postoperative depletion.

Cat 22 shows how completely section of the nerve supply of an adrenal protects the store even against the combined effect of the operation and morphine. All preparations having been made to insure almost simultaneous removal of one adrenal and denervation of its fellow, the right adrenal was excised in 5 minutes, and the nerves of the left adrenal were cut within the next 2 minutes. Even after 8 hours, the epinephrin content of the left adrenal was precisely the same as that of the right; namely, 0.26 mg.

In Table XII are given the results of assays of the adrenals in a number of experiments on the liberation of epinephrin into the blood.⁵ All except the first show a relatively low content, since the nerves were cut in the course of the experiment, usually some hours after the beginning. Where the innervation of one adrenal is divided after several hours of experimentation, so great a depletion of the epinephrin store of both glands has already taken place that subsequent division of the nerves of the other, an hour or two later, does not generally establish such a striking difference between the two glands as when the nerves on one side are severed at the beginning.

In Table XIII are shown the epinephrin assays of the adrenals obtained in other experiments on liberation of epinephrin. In these the innervation of the glands was not interfered with. Equality of content in the two adrenals of the same animal is well shown. While there are, of course, variations in the different animals, the average content is lower than that of the cats in Tables I and II.

TABLE XII.

Depletion of the Epinephrin Store of the Innervated Adrenal during Physiological Experiments.

No. of animal.	Weight of adrenal.		Epinephrin.		Duration of experiment.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	gm.	mg.	mg.	hrs.	
Cat 23	0.260	0.286	0.18	0.25	5	Connections of right semilunar ganglion cut 11 days before experiment.
" 24	0.230	0.230	0.17	0.28	2½	Connections of right semilunar ganglion cut 15 days before experiment. Used for liberation experiment and died during the experiment. In poor condition; abortion the day before.
" 25	0.204	0.211	0.10	0.17	5	Right major and minor splanchnics cut 3½ hrs. after beginning of experiment.
" 26	0.197	0.201	0.14	0.16	5½	Right sympathetic (in thorax) cut 2½ hrs. after beginning of experiment. Left sympathetic cut 2¼ hrs. later.
" 27	0.140	0.140	0.11	0.11	3¾	Right sympathetic (in thorax) cut 3 hrs. after beginning; left sympathetic cut ¾ hr. later.
" 28	0.204	0.216	0.17	0.18	4	Both sympathetics cut in thorax, 2½ hrs. after beginning.
" 29	0.120	0.122	0.18	0.18	2½	Both sympathetics cut 1 hr. after beginning.
" 30	0.221	0.228	0.13	0.14	6	Both sympathetics cut 5 hrs. after beginning. 1 hr. later, adrenals massaged.
" 31	0.242	0.234	0.18	0.16	4½	Both sympathetics cut 3 hrs. after beginning.
" 32	0.185	0.200	0.11	0.09	5½	Both major splanchnics cut 4½ hrs. after beginning.
Dog 16	0.524	0.560	0.41	0.37	6¼	Right sympathetic (in thorax) cut 4½ hrs. after beginning, left sympathetic 1¼ hrs. later.

TABLE XIII.

Epinephrin Content in Cats Used for Experiments under Urethane and Ether without Section of Nerves to Adrenals.

No. of animal.	Weight of adrenal.		Epinephrin.		Duration of experiment.
	Left.	Right.	Left.	Right.	
	gm.	gm.	mg.	mg.	hrs.
33	0.178	0.164	0.14	0.14	5 $\frac{1}{4}$
34	0.294	0.290	0.17	0.16	4
35	0.226	0.224	0.20	0.19	3 $\frac{3}{4}$
36	0.256	0.238	0.20	0.19	4 $\frac{3}{4}$
37	0.330	0.364	0.18	0.20	5 $\frac{3}{4}$

Depletion of Epinephrin Store of the Innervated as Compared with That of the Denervated Adrenal in Animals, Dead of Infections, Etc.

The opportunity of making these observations was afforded by the death, from various causes, of a certain number of animals whose adrenal nerve supply had been cut on one side. The results are given in Table XIV. Complete or almost complete depletion of the epinephrin store of the still innervated gland was far more frequently observed in this group than in any of the experimental groups.

Infections of various kinds (pyogenic in Cats 38 and 41, pneumonia in Dogs 17 and 18) caused particularly marked depletion. It has been stated by a number of observers that the adrenals in animals killed by various infections are poor in epinephrin. Elliott² found a small epinephrin load in persons dead of various septic fevers. The results cited in Table XIV show clearly that this depletion is brought about, in great part at any rate, through the nerves supplying the adrenals. It is not possible to say from these results whether, in addition, there is any direct action of the poisons on the glands.

In a cat (No. 43) dead of distemper the epinephrin content was quite low (0.09 mg.), but in another cat (No. 42), dead of the same disease, the load was fairly high (0.28 mg.), although perhaps not as much as would have been expected in a healthy animal of the same size. The cat was exceptionally large, as were also the adrenals.

TABLE XIV.

Depletion of the Epinephrin Store of the Innervated Adrenal in Animals Dead from Infections, Etc.

No. of animal.	Weight of adrenal.		Epinephrin.		Remarks.
	Left.	Right.	Left.	Right.	
	gm.	gm.	mg.	mg.	
Cat 38.....	0.184	0.191	0.16	Trace.	Connections of left semilunar ganglion cut 8 days before death from infection (peritonitis).
" 39	0.312	0.304	0.09	"	Connections of left semilunar ganglion cut 5 days before death from unknown cause. Cat emaciated and in poor condition before operation.
" 40.....	0.170	0.198	Trace.	0.13	Connections of right semilunar ganglion cut 2 days before death from unknown cause. Cat in same condition as No. 39.
" 41.....	0.308	0.300	0.08	0.14	Connections of right semilunar cut 25 days before death. General infection; abscess in wound in neck and in flank; eyes infected.
" 42	0.396	0.402	0.28	0.28	Died of distemper 14 days after excision of left superior cervical ganglion.
" 43.....	0.278	0.272	0.09	0.09	Died of distemper 6 days after excision of left superior cervical ganglion.
Dog 17.....	0.530	0.570	0.50	0.31	Left major splanchnic cut 13 days before death from pneumonia.
" 18.....	0.360	0.350	0.30	0.08	Left major and minor splanchnics, 2 lumbar ganglia, and connections of celiac ganglion cut 14 days before death from pneumonia.
Rabbit 50.....	0.200	0.210	0.09	Trace.	Left adrenal excised 3 days before death from unknown cause.

The Effect of Electrical Stimulation of the Splanchnic Nerves on the Epinephrin Store.

Elliott has stated that stimulation of the splanchnics by induction shocks for periods varying from 3 to 7 hours produces only a slight effect on the epinephrin content. Stimulation was applied for a few minutes at a time followed by a few minutes of rest. "When the nerve was faradized continuously for a period of 2 hours the loss was almost inappreciable."

Our observations confirm those of Elliott, in as far as they show that it is much more difficult to demonstrate depletion by splanchnic

stimulation than under the influence of ether, operations, etc., despite the fact that more epinephrin is liberated in a given time during appropriate stimulation of the nerves than is liberated under anesthetics without stimulation. The difficulty of showing depletion with splanchnic stimulation is, therefore, not due to the small amount liberated. We cannot see any other explanation than that splanchnic stimulation also increases the rate of accumulation of the epinephrin in the gland.

Continuous stimulation of the splanchnic is not suitable for producing depletion, nor is stimulation for several minutes at a time with intervals of rest. For we have seen in experiments on liberation of epinephrin into the blood that the amount excreted rapidly declines when stimulation is kept up.

However, by stimulating only for a few seconds at a time, with intervals of 1 or 2 minutes' rest, and continuing the experiment over many hours we have been able to demonstrate distinct depletion of the gland whose nerve was excited as compared with the other. The reactions of the denervated eye were used to control the efficiency of the stimulation. A strength and duration of stimulation was chosen which gave good but not maximal dilatation of the pupil, and the rest intervals were increased or diminished from time to time, so as to keep each stimulation effective.

In Table XV is given a condensed protocol of an experiment on a cat in which over 300 successful stimulations of the right sympathetic trunk (with the splanchnic) in the thorax were made. The amount of epinephrin liberated by each stimulation was estimated by determining, at different times throughout the experiment, the amount of adrenalin which had to be injected to give approximately a pupil dilatation of the same magnitude, and beginning at the same time interval, as the dilatation caused by the liberated epinephrin. Both sympathetics were divided simultaneously in the thorax, so that the glands were under equal conditions, except for stimulation of the nerves of the right. At the end of the experiment the left adrenal weighed 0.159 gm. and contained 0.20 mg. of epinephrin, and the right adrenal weighed 0.181 gm. and contained 0.08 mg. of epinephrin.

The assays by adrenalin solution of the amount of epinephrin liberated from the right adrenal into the blood yielded the following results.

Epinephrin liberated by 316 stimulations, 0.31 mg.

Time.	No. of stimulations.	Adrenalin corresponding to each stimulation.	Total epinephrin.
		mg.	mg.
3.10-5.01 p.m.....	47	0.002	0.09
5.04-7.28 p.m.....	95	0.0013	0.12
7.32 p.m.-12.15 a.m.....	174	0.0006	0.10

With the short time of each stimulation and the relatively long intervals of rest, it is to be supposed that the power of the adrenal to respond to stimulation of its nerves was conserved as much as possible. Nevertheless, the diminution in the response, which was not apparent for a long time during the earlier part of the experiment became more marked as time went on. This is strikingly illustrated in the table showing the amount of liberated epinephrin. The 174 stimulations in the last period yielded only 0.10 mg. of epinephrin, while forty-seven stimulations of the same duration (5 seconds) yielded in the first period of the experiment 0.09 mg. Add to this the fact that the strength of stimulation had to be considerably increased in the latter part of the experiment, as shown by the diminished distance between the coils given in the protocol.

The load of the right adrenal at the time the nerves were cut may be taken at 0.20 mg., the same as that of the left. This represents a good load for these small adrenals (the cat weighed only a little over 2 kilos), especially as some loss must have occurred in the hour after the administration of urethane before the nerves were divided. The right adrenal gave off to the blood 0.31 mg. of epinephrin, of which 0.12 mg. could have come from the store. This is on the assumption that no epinephrin whatever is liberated in the absence of innervation.⁵ An amount approximately equal to the initial load must have been formed in the time the experiment lasted.

In other experiments lasting for a shorter time we have not been able to obtain evidence of depletion of the store by splanchnic stimulation. In one with over fifty stimulations, each of which caused the liberation of enough epinephrin to elicit good eye reactions, no deficiency in the store of the stimulated gland was found, although in the 4 hours of the experiment an amount of epinephrin not less than the maximal load of a single gland in the cat had been given off by it to the blood.

It is possible that in prolonged experiments the capacity of the adrenal to form epinephrin at an increased rate in response to splanchnic stimulation is lessened, and that this is the reason why depletion of the store can then be demonstrated.

TABLE XV.

Condensed Protocol. Cat 44, Weight 2.25 Kilos. Superior Cervical Ganglion Excised 17 Days before. Animal Pregnant. Cannulas in Right Femoral Vein and Trachea. Unless Otherwise Mentioned, Time of Stimulation Was Always 5 Seconds. The Left Sympathetic Was Stimulated Only Once.

Time.		Interval between beginning of stimulation and of pupil dilatation.
<i>p. m.</i>		<i>sec.</i>
1.40.....	Urethane, 3 gm. by stomach tube.....	
2.20.....	A little ether. No more ether during experiment....	
2.45.....	Both sympathetics (with splanchnic) ligated and cut in thorax.....	
3.10.....	Stimulated left sympathetic (11 cm. between coils)...	9.6
3.30-4.08.....	Stimulated right sympathetic (3-5 sec.) (9-10 cm.) 10 times.....	6 6-7.8*
4.08.....	Adrenalin injections. Same pupil reactions given by 0.3 cc. (1 : 140,000)†	
4.09-4.27.....	Stimulated right sympathetic (9 cm.) 15 times....	6.0-7.2
4.27.....	Adrenalin injections. Reaction, 0.3 cc.....	
4.28-4.36.....	Stimulated right sympathetic (9 cm.) 6 times.....	7.4-8.8
4.36.....	Adrenalin injections. Reaction, 0.3 cc.....	
4.40-4.46.....	Stimulated right sympathetic (9 cm.) 5 times.....	7.6-8.2
4.47.....	Adrenalin injections. Reaction, 0.3 cc.....	
4.49-5.01.....	Stimulated right sympathetic (9 cm.) 11 times.....	6.8-7.8
5.02.....	Adrenalin injections. Reaction, 0.3 cc.....	
5.04-5.21.....	Stimulated right sympathetic (9 cm.) 14 times.....	7.2-8.0
5.22.....	Adrenalin injections. Reaction, 0.25 cc.....	

* The time interval of the pupil reaction was constant throughout the experiment. Only the minimum and maximum times are given in this column for the various series of stimulations. The times for the pupil reactions yielded by these 10 stimulations may be given as an example (7.4, 7.0, 7.4, 7.8, 7.8, 7.2, 7.2, 7.6, 6.6, 7.2). The number in parenthesis after stimulation gives the distance between the coils in cm.

† Only the quantities of adrenalin found in the trial injections to correspond with the effects of the splanchnic stimulations are reproduced from the protocol. The adrenalin solution was itself assayed. The corrected concentration is what is given in the protocol. A freshly made solution in 0.9 per cent sodium chloride was used for each assay. The adrenalin solution injected was always a 1:140,000. The concentration of the solution is therefore henceforth omitted in the protocol.

TABLE XV—*Concluded.*

Time.		Interval between beginning of stimulation and of pupil dilatation.
<i>p. m.</i>		<i>sec.</i>
5.25-5.36.....	Stimulated right sympathetic (9 cm.) 8 times.....	7.2-8.2
5.38.....	Adrenalin injections. Reaction, 0.2 cc.....	
5.40-5.59.....	Stimulated right sympathetic (9 cm.) 15 times.....	6.6-8.6
6.00.....	Adrenalin injections. Reaction, 0.2 cc.....	
6.02-6.18.....	Stimulated right sympathetic (9 cm.) 13 times.....	6.8-7.4
6.20.....	Adrenalin injections. Reaction, 0.2 cc.....	
6.22-6.36.....	Stimulated right sympathetic (9 cm.) 12 times.....	6.4-7.2
6.40.....	Adrenalin injections. Reaction, 0.2 cc.....	
6.42-6.53.....	Stimulated right sympathetic (9 cm.) 7 times.....	7.0-7.4
6.58.....	Adrenalin injections. Reaction, 0.2 cc.....	
7.00-7.28.....	Stimulated right sympathetic (9 cm.) 26 times.....	6.6-7.2
7.31.....	Adrenalin injections. Reaction, 0.2 cc.....	
7.32-7.50.....	Stimulated right sympathetic (9 cm.) 18 times.....	6.2-7.2
7.54.....	Adrenalin injections. Reaction, 0.1 cc.....	
7.55-8.10.....	Stimulated right sympathetic (8 cm.) 13 times.....	6.2-8.8
8.12.....	Adrenalin injections. Reaction, 0.1 cc.....	
8.15-8.38.....	Stimulated right sympathetic (8 cm.) 20 times.....	6.4-7.8
8.40.....	Adrenalin injections. Reaction, 0.1 cc.....	
8.42-9.20.....	Stimulated right sympathetic (7.5 cm.) 26 times.....	6.4-7.8
9.22.....	Adrenalin injections. Reaction, 0.1 cc.....	
9.26-9.48.....	Stimulated right sympathetic (7.5 cm.) 14 times.....	6.8-8.0
9.50.....	Adrenalin injections. Reaction, 0.1 cc.....	
9.53-10.25.....	Stimulated right sympathetic (7.5 cm.) 24 times.....	6.8-7.8
10.27.....	Adrenalin injections. Reaction, 0.1 cc.....	
10.30-10.50.....	Stimulated right sympathetic (7.5 cm.) 16 times.....	6.4-7.8
10.53.....	Adrenalin injections. Reaction, 0.1 cc.....	
10.55-11.30.....	Stimulated right sympathetic (7.5 cm.) 23 times.....	6.8-8.8
11.34.....	Adrenalin injections. Reaction, 0.1 cc.....	
11.37 p.m.-12.15 a.m.	Stimulated right sympathetic (7.5 cm.) 20 times.....	6.8-9.0
12.15 a. m.....	Adrenalin injections. Reaction, 0.1 cc.....	

Circulation good up to the end of the experiment; the heart was beating well, although the pulse was gradually becoming more rapid toward the last hour of the experiment.

The left adrenal weighed 0.159 gm. and contained 0.20 mg. of epinephrin.

The right adrenal weighed 0.181 gm. and contained 0.08 mg. of epinephrin.

SUMMARY.

1. No evidence has been obtained that in cats and dogs with the nerves of one adrenal cut, emotional disturbances cause depletion of the epinephrin store of the normally innervated adrenal as compared with its fellow.

2. The depletion of the epinephrin store in cats under morphine is not dependent upon so called morphine fright, since a similar depletion is found in dogs in which, as is known, morphine produces symptoms the reverse of those of fright.

3. The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the nerve supply of the other cut, and in which accordingly no detectable liberation of epinephrin takes place.

4. The reactions of the denervated iris elicited by emotional disturbance, asphyxia, or etherization in a cat, one of whose adrenals has been removed and the nerves of the other cut, do not differ from these reactions in cats whose adrenals have not been interfered with.

5. The influence of postoperative edema of the adrenal in diminishing the epinephrin load, and the recuperation of the load after a time, have been studied in rabbits.

6. The diminution in the epinephrin store of the adrenals which follows operations on animals (postoperative depletion) has been studied. It is only in part associated with the anesthesia, since it may be as marked 6 or 8 hours after an operation lasting less than 1 hour as after 6 or 8 hours' anesthesia without operation.

7. One adrenal was removed in rabbits and the epinephrin content of the remaining gland assayed at varying periods of time after removal of the first, the periods being longer than the time necessary for recovery from the postoperative depletion. In general, the second adrenal contained more epinephrin than the first, sometimes double the amount.

8. Marked depletion of the epinephrin store of innervated adrenals as compared with the corresponding denervated glands was seen in animals dead of infections of various kinds.

9. As shown by Elliott, diminution of the stock of epinephrin in the adrenal through electrical stimulation of the splanchnics is not easy to demonstrate, despite the fact that the liberation of epinephrin into the blood is notably increased by the stimulation. With short periods of stimulation, however, repeated over a long time at intervals just long enough to prevent fatigue, it has proved possible to demonstrate a distinct depletion.

49 (1227)

The influence of certain conditions on the rate at which epinephrin is liberated from the adrenals into the blood.

By G. N. STEWART and J. M. ROGOFF.

[From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University, Cleveland, Ohio.]

1. By means of the rabbit intestine and uterus segment tests, we have obtained further evidence that, under our experimental conditions at any rate, the rate of discharge of epinephrin into the blood of the adrenal veins is relatively steady and not easily influenced by such procedures as we have tried; for example, stimulation of the afferent fibers in large peripheral nerves (sciatic and brachial) or asphyxia. This is not because the discharge is already maximal owing to the necessary conditions of the experiment (trauma, anesthesia, etc.). For, by electrical stimulation of the cut splanchnic, the rate of liberation can be made decidedly greater than the rate of spontaneous liberation with intact splanchnics.

2. Unlike the rate of liberation per unit of time, the concentration of epinephrin in the adrenal vein blood can be observed to vary decidedly in the course of an experiment, increasing, in general, as the rate of blood flow decreases. This can be shown by collecting adrenal vein blood in successive samples. When the blood flow slackens, owing to hemorrhage or other circumstances, the earlier specimens will be found to contain a smaller concentration of epinephrin than the later specimens.

For example, in a dog, weighing 10 kg., the first sample from the cava pocket, into which the adrenal veins were alone discharging, flowed at the rate of 8 c.c. per minute, the second sample 7.2 c.c., third 5.8 c.c., fourth 4.4 c.c., fifth 3.2 c.c., sixth 2.4 c.c., seventh 1.5 c.c. A definite increase in the epinephrin concentration in the successive samples was clearly shown by the intestine and especially by the uterus tests. The concentration was assayed in the first sample at somewhat more than 1 : 3,300,000; in the third sample at somewhat more than 1 : 1,670,000; in the seventh sample at somewhat less than 1 : 750,000.

The increase in the concentration in the blood is far too great to be accounted for by any increase in the relative proportion of plasma to corpuscles associated with hemorrhage without change in the concentration of epinephrin in the plasma. And it has been demonstrated that the sera separated from the successive samples of blood show a progressively increasing concentration of epinephrin.

Even when the circulation through the adrenals is stopped altogether by clamping the veins, the liberation of epinephrin into the pent-up blood continues for a time at an apparently undiminished rate and the concentration of epinephrin in the blood must go on increasing.

3. For the reason mentioned in paragraph 2 it is not in general permissible to deduce changes in the rate of liberation of epinephrin from changes in its concentration in the adrenal vein blood, unless the rate of blood flow through the adrenals is known. Changes in the concentration of epinephrin in the blood of the inferior cava above the adrenals can be produced by alterations in the rate of blood flow in the cava, even where the rate of liberation of epinephrin from the adrenals has remained constant.

4. No evidence has been obtained that after section of the nerves of one adrenal, any compensatory increase in the rate of liberation of epinephrin from the other gland occurs. The fact that section of one splanchnic diminishes the discharge of epinephrin by a half, without causing any material fall of blood pressure, affords additional evidence that the epinephrin discharged by the adrenal veins is not directly a factor, or at least not an important one in maintaining the blood pressure.

50 (1228)

The proportion in which adrenalin distributes itself between corpuscles and serum in relation to the technique of testing for epinephrin in blood.

By G. N. STEWART and J. M. ROGOFF.

[From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University, Cleveland, Ohio.]

1. When adrenalin was added to defibrinated blood, and the blood centrifuged after an hour, the serum was found by the colorimetric method of Folin, Cannon and Denis, to contain practically the whole of the added adrenalin.

3 c.c. adrenalin solution (Parke, Davis & Co.), corresponding to 2.64 mg. epinephrin when assayed colorimetrically, was added to 30 c.c. cat's defibrinated blood. Correcting for the small amount of color given by the serum itself in the test, the amount of adrenalin found in 10 c.c. of the serum separated from the adrenalin blood corresponded to 1.37 mg. epinephrin. The proportion by volume of serum in the blood was 62 per cent. The amount of serum in 30 c.c. of the adrenalin blood would, therefore contain $1.37 \times 30 \times 62/100 = 2.55$ mg. adrenalin, *i. e.*, all the adrenalin added was in the serum.

2. The same result was obtained by assaying the adrenalin in the serum by injection into a pithed cat (method of Elliott). The serum gave a rise indicating, when compared with that given by a known amount of adrenalin in control serum, that 10 c.c. of it contained 1.32 mg. adrenalin. This compares with 1.37 mg. by the colorimetric method. The adrenalin blood gave a rise of blood pressure less than that given by the serum and corresponding to the concentration of adrenalin in it. The sediment, which of course contained a very small proportion of serum, gave no measurable rise.

3. Similar results were obtained (with dog's blood to which adrenalin had been added) on segments of rabbit's intestine and uterus. The sediment gave a small inhibition of the intestine and a small increase of tone of the uterus as compared with the serum. The effect of the adrenalin blood was intermediate in amount.

4. The distribution of the naturally secreted epinephrin in the blood from the adrenal veins (of the dog) was also investigated with the same result. Only the rabbit intestine and uterus were employed, the other methods not being sufficiently sensitive for the small concentrations found in blood. In one experiment the concentration of epinephrin in the blood was assayed at 1:8,000,000, in the serum at 1:3,000,000. The sediment gave practically nothing. It so happened that the blood used was extremely rich in corpuscles, a circumstance favorable rather than otherwise for testing the point in question, as the serum would be more than ordinarily rich in epinephrin as compared with the blood, if all the epinephrin is contained in the plasma. The proportion of serum by volume in the blood was 36 per cent. On the hypothesis that all the epinephrin was in the serum, this would give $1 : 100/36 \times 3,000,000$, *i. e.*, 1:8,300,000 as the concentration in the blood.

5. When search is being made for the minute quantities of epinephrin present in blood, serum (or plasma) should, in general, be preferred to blood in making the tests.

THE PROPORTION IN WHICH ADRENALIN DISTRIBUTES ITSELF BETWEEN CORPUSCLES AND SERUM IN RELATION TO THE TECHNIQUE OF TESTING FOR EPINEPHRIN IN BLOOD¹

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In using such biological test-objects as rabbit intestine and uterus segments, etc., for the detection of epinephrin, it is desirable to know how the epinephrin is distributed between the plasma (or serum) and corpuscles. For the concentrations of epinephrin in blood being always small, it would generally be advantageous to employ the fraction, supposing the distribution is not uniform, in which the greatest concentration exists.

We began by adding adrenalin (Parke, Davis and Company) to defibrinated blood, allowing the blood to stand for a time and then centrifuging it. The blood, serum and sediment were then tested on rabbit intestine and uterus segments.

Experiment 1. 0.5 cc. of adrenalin solution (Parke, Davis and Company) was added to 100 cc. of dog's blood. A portion of the mixture was centrifuged and clear serum free from erythrocytes obtained. The adrenalin solution assayed by the colorimetric method of Folin, Cannon and Denis as equivalent to a 1:1660 solution of epinephrin. In order to obtain a decided effect the adrenalin was purposely added in such amount as would give a concentration larger than that in which the naturally secreted epinephrin exists even in adrenal vein blood. Some of the dog's blood without addition of adrenalin was also centrifuged and the defibrinated blood, serum and sediment were used for the segment tests.

¹ A preliminary account of the work was given at the joint meeting of the Federation of American Societies for Experimental Biology, December 28, 1916. A note was published in the Proceedings of the Society for Experimental Biology and Medicine, January 17, 1917.

It will be seen from figure 1² that the inhibitory effect of the serum separated from the adrenalin blood upon the rabbit intestine segments is very much greater than that of the sediment. The sediment indeed has an action so slight as to suggest that it is due solely to the serum still unavoidably present between the corpuscles. The effect of the defibrinated blood is intermediate in magnitude between that of the serum and sedi-

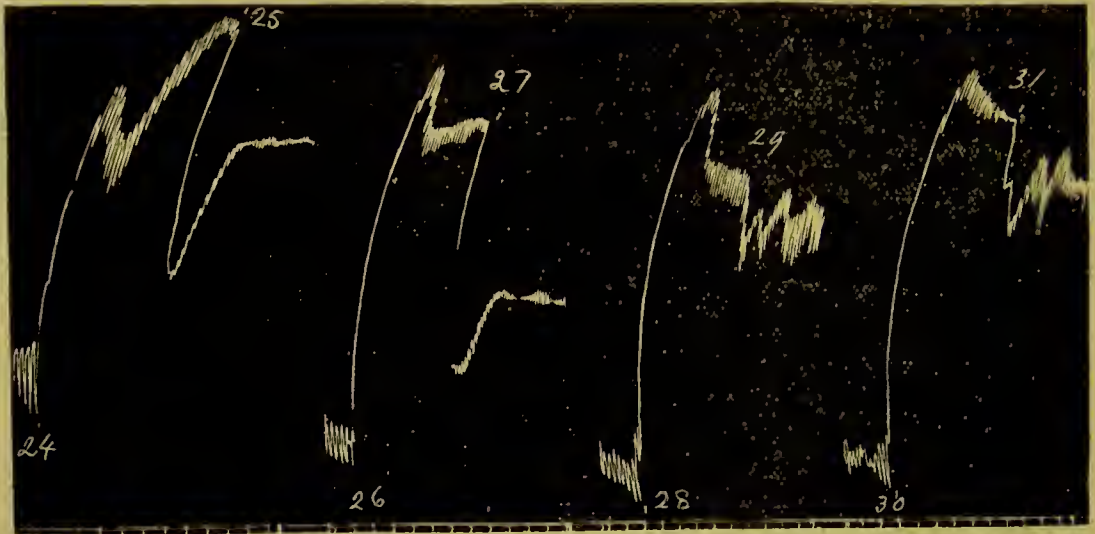


FIG. 1. ACTION OF ADRENALIN DEFIBRINATED BLOOD, SERUM AND SEDIMENT (EXPERIMENT 1) ON INTESTINE SEGMENTS

At 24, Ringer was replaced by defibrinated blood, and this at 25 by adrenalin defibrinated blood, diluted with 3 volumes of defibrinated blood. At 26, Ringer was replaced by serum, and this at 27 by adrenalin serum diluted with 3 volumes serum. At 28, Ringer was replaced by ordinary sediment, and this at 29 by sediment of the adrenalin blood, diluted with 3 volumes of the indifferent sediment. The inhibitory effect at 29 was insignificant; even when undiluted adrenalin sediment replaced indifferent sediment at 31 the effect was small, compared with that of the diluted adrenalin serum or blood.

ment. The observations were repeated several times throughout the experiment, always with the same result.

The same thing is even better seen in the uterus segment tracings (fig. 2). The uterus being very sensitive to adrenalin, it was necessary to greatly dilute the liquids tested in order to

² The tracings in all the figures except figures 15 and 16 have been reduced to one-half. Figure 15 is not reduced. Figure 16 is reduced to two-thirds. Time trace half minutes.

obtain a comparison. For instance, the adrenalin serum when diluted (with Ringer's solution) in the proportion of 1 in 6 (observation 42, fig. 2) gave a maximal effect, as was the case also with a dilution of 1 in 11 (observation 43). With a dilution of 1 in 16 (observation 44) the effect was still nearly maximal,

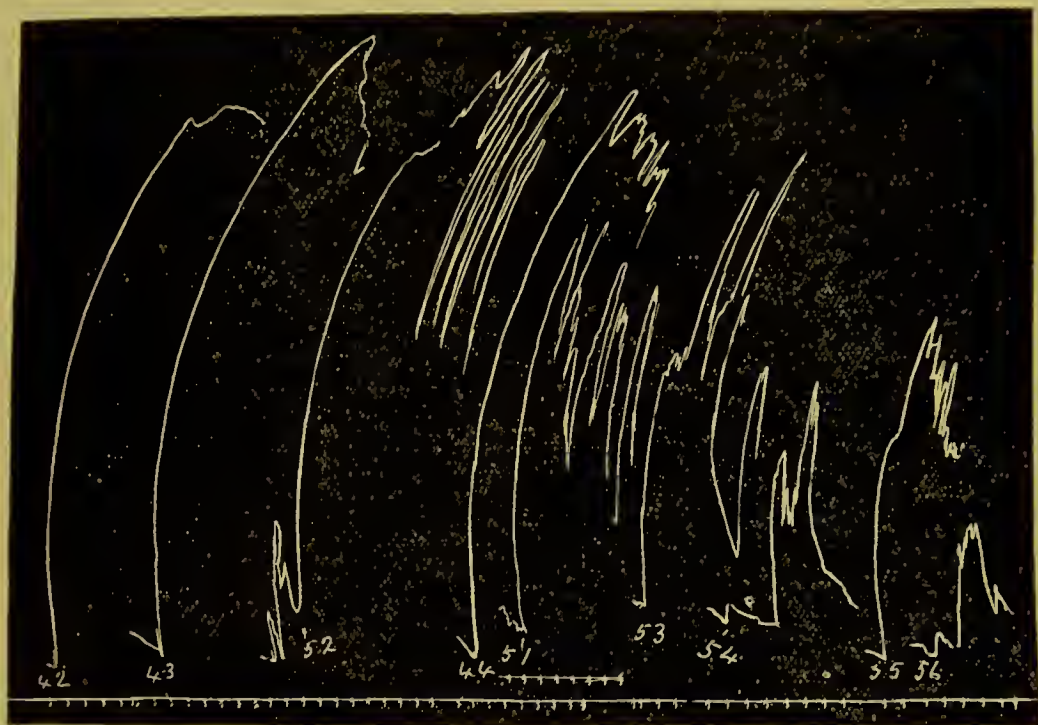


FIG. 2. ACTION OF ADRENALIN DEFIBRINATED BLOOD, SERUM AND SEDIMENT (EXPERIMENT 1) ON THE UTERUS SEGMENTS

At 42, Ringer was replaced by adrenalin blood serum (1 in 6), at 43 by the same serum (1 in 11), at 44 by the same serum (1 in 16), at 52 by the same serum (1 in 24). At 51, Ringer was replaced by adrenalin blood (1 in 24), at 53, by adrenalin blood sediment (1 in 6), at 54, by the adrenalin blood sediment (1 in 24), at 55 by the ordinary defibrinated blood (1 in 6), at 56, by the ordinary defibrinated blood (1 in 24).

except that the rhythmical contractions were not so completely suppressed by the great increase of tone. Observation 52 shows the effect of adrenalin serum in the dilution 1 in 24. The increase of tone is much greater than that produced by the adrenalin blood similarly diluted (observation 51). The sediment from the adrenalin blood in the dilution 1 in 24 (observation 54)

gives an effect far smaller than that of the adrenalin blood in the same dilution and not very much greater than that of the ordinary defibrinated blood (diluted 1 in 24) (observation 56). Even in dilution 1 in 6, the adrenalin sediment causes a much smaller rise (observation 53) than the serum in dilution 1 in 24.

This experiment, then, shows that the fraction of the adrenalin blood containing the erythrocytes, with only a minimal amount of serum, gives both with the intestine and with the uterus, adrenalin effects far inferior to those given by the adrenalin blood, and that the action of the adrenalin blood is markedly less than that of the serum.

The serum made up 52.3 per cent of the blood as estimated by the electrical method.³ By the haematocrit the amount of serum was 48 per cent after five minutes rotation, 49 per cent after ten minutes, 50 per cent after fifteen minutes. The haematocrit was turned 4000 times a minute. While the haematocrit may give results which are useful for comparative purposes, it is well known that even when undiluted blood is used, the results are not very accurate so far as the absolute proportion of serum to corpuscles is concerned, since the sediment goes on diminishing for a long time. Nevertheless, in our observations the haematocrit readings were useful for checking up the results given by the electrical method, since the latter gave a proportion of serum which the haematocrit readings more and more nearly approached the longer the period of rotation. The sediment contained 5 per cent of serum, estimated by the electrical method.

With the relatively large quantity of adrenalin added to the blood, the concentration in the serum was so great that a maximal effect was produced on the intestine segments. Therefore considerable dilution would have been necessary to make anything like an accurate assay. However, from such observations as were made, it was shown that the concentration of adrenalin in the serum must have been greater than 1:330,000, and less than 1:110,000. If all the added adrenalin was in the serum, the concentration would be 1:175,000. If the adrenalin was uniformly distributed over corpuscles and serum, the concentration in the serum would be 1:330,000.

³ Stewart, G. N., *Journ. Physiol.*, 1899, xxiv, 356.

At 16 (fig. 3), Ringer was replaced by indifferent serum, and this at 17 by the serum from the adrenalin blood. The great inhibition carried the lever 2 cm. below the drum. At 8, Ringer was replaced by indifferent serum, and this at 9 by serum containing 1:330,000 adrenalin. It is obvious that the inhibition at 17 is greater than that of 9. Therefore the adrenalin blood serum contains more than 1:330,000 adrenalin. At 18, Ringer was replaced by the indifferent serum, and this at 19 by serum from the adrenalin blood diluted with two volumes of the indifferent serum. The inhibition was less than at 9, there-

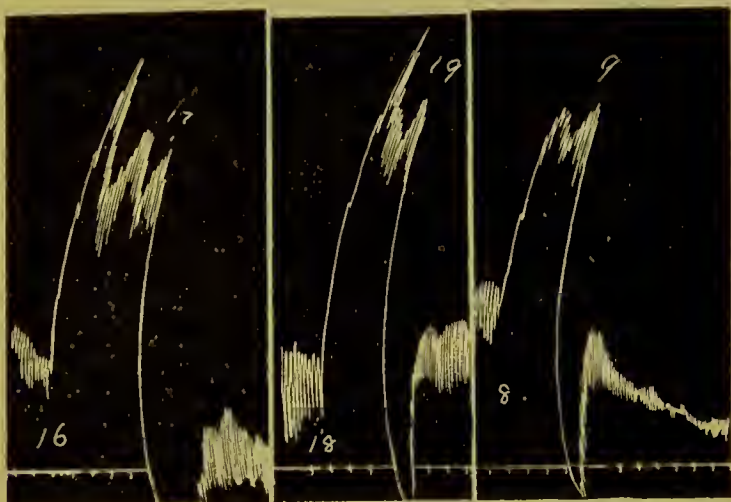


FIG. 3. COMPARISON OF SERUM FROM ADRENALIN BLOOD (EXPERIMENT 1) WITH SERUM TO WHICH A KNOWN AMOUNT OF ADRENALIN HAD BEEN ADDED

For description see text

fore the adrenalin blood serum contained less than 1:110,000 adrenalin. The fact that the sediment exerts so small an adrenalin action compared with the serum or blood is of itself almost a quantitative proof that practically all the adrenalin which is effective in the segment reactions is in the serum. It might be objected, however, that a material fraction of the added adrenalin had entered the corpuscles, but that being bound in some way within them and not being able freely to pass out of them it could not exert its action on the segment. Experiment 2 demonstrates that there is no foundation for this suggestion.

In experiment 2 adrenalin was added to cat's blood and its concentration in the serum determined by the colorimetric method and by injection into a pithed cat (Elliott's method). Adrenalin blood and sediment were also tested on the pithed cat.

Experiment 2. 3 cc. adrenalin solution (Parke, Davis and Company), corresponding to 2.54 mgm. epinephrin (as assayed colorimetrically) was added to 30 cc. cat's defibrinated blood. The blood was allowed to stand for one and one-fourth hours and then centrifuged.

	mgm. epinephrin
10 cc. of the serum gave color equivalent to.....	1.47
Color given by the ordinary serum in the test equivalent to.....	0.09

	1.38
Added 1 cc. adrenalin solution, corresponding to 0.88 mgm. epinephrin, to 10 cc. serum. The color in the test was equivalent to.	0.96
Color due to the serum.....	0.09

	0.87

There was therefore practically no loss of adrenalin in the serum in the test. The defibrinated blood contained 62.5 per cent of serum by the electrical method; by the haematocrit 57 per cent after eight minutes' rotation, 60.5 per cent after sixteen minutes, 62 per cent after twenty-one minutes.

Taking the amount of serum as 62 per cent, 30 cc. of blood would contain 18.6 cc. of serum. The amount of adrenalin found in 18.6 cc. of serum would be $1.38 \times 1.86 = 2.56$ mgm., i.e., all the adrenalin added was in the serum.

Specimens of the blood pressure tracings from the pithed cat are given in figures 4 to 6. The material used was the same as that used for the colorimetric estimation. Observation 4 (fig. 4) shows the rise produced by injection of 0.1 cc. of the serum separated from the adrenalin blood made up with the ordinary serum to 0.3 cc. The rise is the same as that caused in observation 3 by 0.3 cc. of serum containing 0.0132 mgm. adrenalin.⁴ 0.1 cc. of the serum separated from the adrenalin blood therefore

⁴ The adrenalin solution in serum was made by adding to 10 cc. of the serum 1 cc. of adrenalin solution (Parke, Davis and Company) which assayed by the colorimetric method as equivalent to 0.88 mgm. epinephrin.

contains 0.0132 mgm. adrenalin, that is, 10 cc. of the adrenalin blood serum contains 1.32 mgm. The colorimetric method

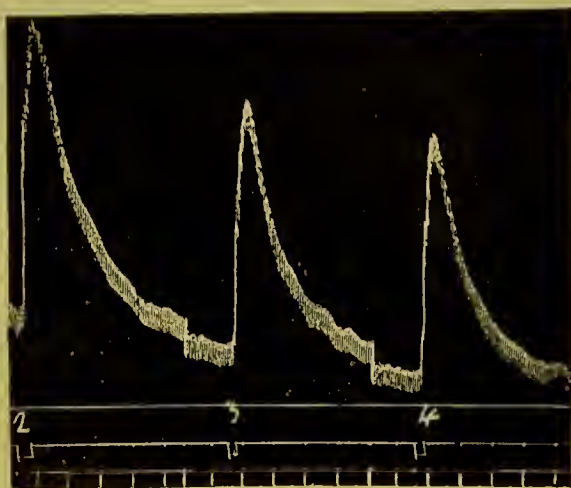


FIG. 4. SPECIMENS OF BLOOD PRESSURE TRACINGS USED IN ASSAY OF ADRENALIN IN SERUM OF BLOOD TO WHICH ADRENALIN HAD BEEN ADDED. (EXPERIMENT 2)

The line of zero pressure has been moved up 30 mm. Signal line above time trace shows time spent in each injection and washing in with Ringer. For further description see text.

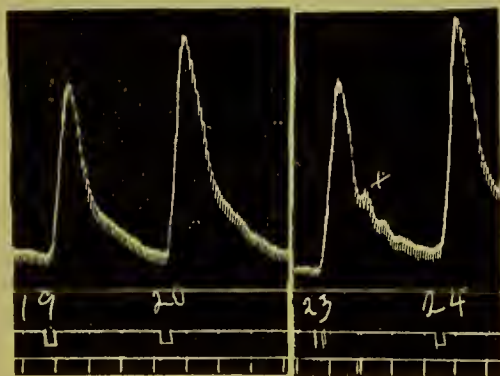


FIG. 5. COMPARISON OF EFFECT PRODUCED ON BLOOD PRESSURE OF PITHED CAT, BY SERUM OF BLOOD TO WHICH ADRENALIN HAD BEEN ADDED AND BY THE BLOOD ITSELF

The line of zero pressure has been moved up 15 mm. 19, blood; 20, serum; 23, blood; 24, serum. At the point marked with a cross the drum jerked forward a little. For further description see text.

gave 1.38 mgm. Observation 2 shows the effect of injection of 0.3 cc. of serum containing twice as much adrenalin as the serum injected in observation 3. At 19 (fig. 5) 0.2 cc. of the

adrenalin blood was injected, and at 20 the same amount of serum from the adrenalin blood. The greater effect of the serum is evident. At 23 the same amount of adrenalin blood, but made up to 0.4 cc. with the indifferent blood, was injected. Observation 24 gives the effect of injection of 0.2 cc. of serum from the adrenalin blood diluted with an equal volume of the indifferent serum. Observation 21 (fig. 6) shows the effect of injection of 0.2 cc. of sediment from the adrenalin blood. As the thick sediment went in very slowly, another observation (22) was made, in which 0.2 cc. of the adrenalin blood sediment, diluted with an equal volume of the ordinary blood, was injected. The rise of pressure was scarcely more than that produced by the injection of 0.5 cc. of the indifferent serum (observation 13).

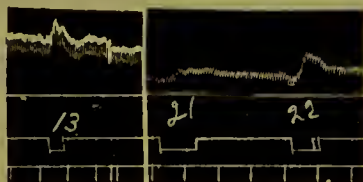


FIG. 6. BLOOD PRESSURE TRACINGS FROM PITHED CAT

Showing the small effect produced by injection of sediment from blood, to which adrenalin had been added (observations 21 and 22). Observation 13, indifferent serum injected. Line of zero pressure moved up 15 mm. For further description see text.

Such experiments show clearly that commercial adrenalin chlorid when added to defibrinated blood remains in the serum without being taken up by the corpuscles, at least for the moderate periods of contact employed. Experiments 3 and 4 were intended to test the question whether the same is true for the naturally secreted epinephrin. It was conceivable that it might exist in a different condition in the blood, which would allow it to distribute itself differently over the corpuscles and plasma. It has been assumed, for example, that the permeability of the erythrocytes for a given salt depends upon their permeability for both ions. If one ion can enter while the other can not, then in general the salt is not taken up by the corpuscles.

Experiment 3 was a qualitative experiment, designed to show, first of all, whether the same difference between sediment and

serum of adrenal vein blood existed as had been demonstrated for the serum and sediment of blood to which adrenalin and been added. A dog, weighing 14.9 kg., was anesthetized with ether,

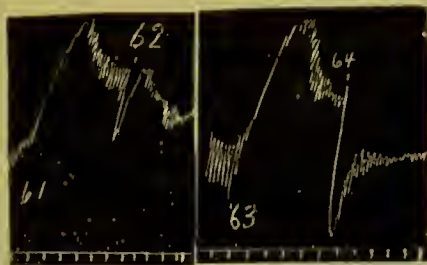


FIG. 7. RABBIT INTESTINE SEGMENT

At 61, Ringer replaced by indifferent blood, and this at 62 by the sediment of dog's adrenal vein blood. At 63, Ringer was replaced by the indifferent blood, and this at 64, by the serum of the adrenal vein blood. All the bloods and sera were diluted with 3 volumes Ringer.

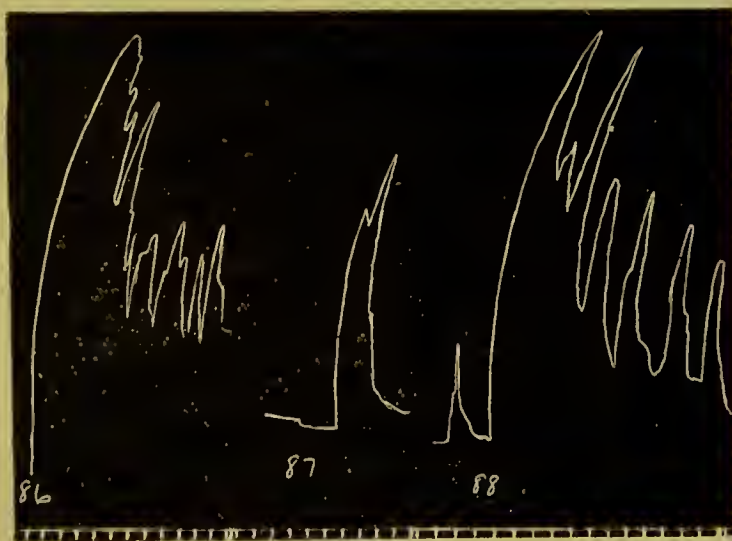


FIG. 8. RABBIT UTERUS SEGMENT

At 86, Ringer was replaced by serum of the dog's adrenal vein blood, used on the intestine segment in figure 7. The serum was diluted with 14 volumes Ringer. At 87, sediment from the adrenal vein blood, and at 88, the blood itself, in each case diluted with 14 volumes Ringer, replaced Ringer.

and pure adrenal vein blood collected in successive samples from a cava pocket. In figures 7 and 8, specimens of the results obtained with one of the samples (the fifth) on the rabbit in-

testine and uterus respectively, are reproduced. The sample consisted of 22.9 cc. of blood collected during three minutes, fifty-five seconds. It was proved *post mortem* that only adrenal vein blood could enter the pocket. It will be seen (fig. 7) that the sediment of the blood (observation 62) produces a far smaller inhibition of the intestine segment than the serum (observation 64). The uterus segments show the same marked difference. Figure 8 is an example. The rise of tone caused by the serum of the same sample of adrenal vein blood (observation 86) is much greater and better sustained than that caused by the sediment (observation 87). The blood gave a rise (observation 88) intermediate in amount, especially as regards the manner in which it was sustained. The three specimens were diluted with 14 volumes Ringer, but the segment was so sensitive that even in this dilution both blood and serum produced about the maximum effect, as regards the immediate increase of tone, and greater dilution would have been required to bring out a difference in this respect between them.

The next experiment (experiment 4) was planned to afford as accurate as assay as possible of the epinephrin concentration in adrenal vein blood. Since the possible concentration is not sufficiently great for quantitative estimation by the colorimetric method, we used only the rabbit intestine and uterus segments, making a large number of comparisons with indifferent blood and serum to which known amounts of adrenalin had been added.

Experiment 4. Dog, body weight, 16.45 kg. Ether anesthesia. 200 cc. of blood was first collected from the external jugular vein. Then a cannula was inserted into the left iliac vein, and a cava pocket tied off so as to collect pure adrenal vein blood. It was verified *post-mortem* that the pocket received no blood except from the adrenals. Blood was collected from the pocket in four successive samples during forty minutes. During this time, the blood flowed without interruption from the cannula draining the pocket. Portions of the various specimens were centrifuged and serum obtained.

Of the considerable number of observations made to estimate the limits of the epinephrin concentration, as sharply as possible,

typical specimens are shown in figures 9 to 15. In figure 9 Ringer's solution was replaced at 11 by jugular blood diluted with 2 volumes Ringer, and this at 12 by blood of the second pocket specimen similarly diluted. At 13 Ringer was replaced by sediment of indifferent (jugular) blood diluted with 2 volumes Ringer, and this at 14 by sediment of the second pocket specimen similarly diluted. The difference in the inhibitory effects of the blood and sediment is very striking.

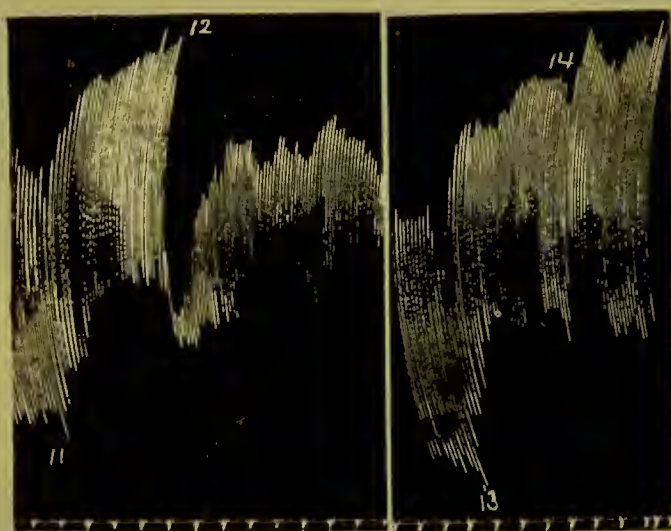


FIG. 9. COMPARISON OF EFFECT OF ADRENAL VEIN BLOOD AND ITS SEDIMENT ON RABBIT INTESTINE SEGMENTS

11, Ringer replaced by indifferent blood, and this at 12 by adrenal vein blood. 13, Ringer replaced by sediment of indifferent blood, and this at 14 by sediment of adrenal vein blood. The bloods and sera were diluted with 2 volumes Ringer.

In figure 10, at 17, Ringer's solution was replaced by serum of jugular blood diluted with 4 volumes Ringer, and this at 18 by serum of the second pocket specimen diluted to the same degree. At 19 Ringer was replaced by jugular blood, diluted with 4 volumes Ringer, and this at 20 by blood of the second pocket specimen similarly diluted. It will be noted that the effect of the serum is much greater than that of the blood. Indeed, by referring to figure 9, it will be seen that the inhibitory effect of the serum four times diluted is greater than that of the blood twice diluted, i.e., the serum contains a concentration of epinephrin more than twice as great as the blood.

Figures 11 to 14 give specimens of tracings taken, with rabbit intestine segments, to assay the concentration of epinephrin in the adrenal vein blood and serum by comparing their effects

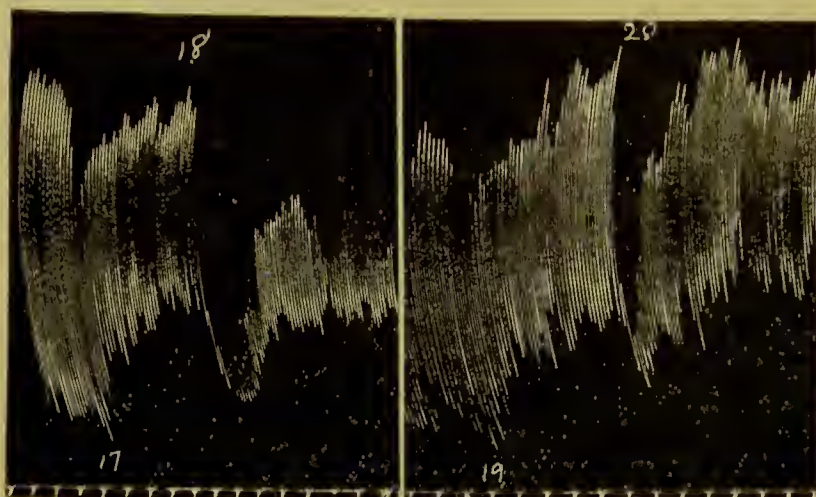


FIG. 10. RABBIT INTESTINE SEGMENT

At 17, Ringer replaced by indifferent serum, and this at 18 by adrenal vein serum. At 19, Ringer replaced by indifferent blood, and this at 20 by adrenal vein blood. The bloods and sera were diluted with 4 volumes Ringer.

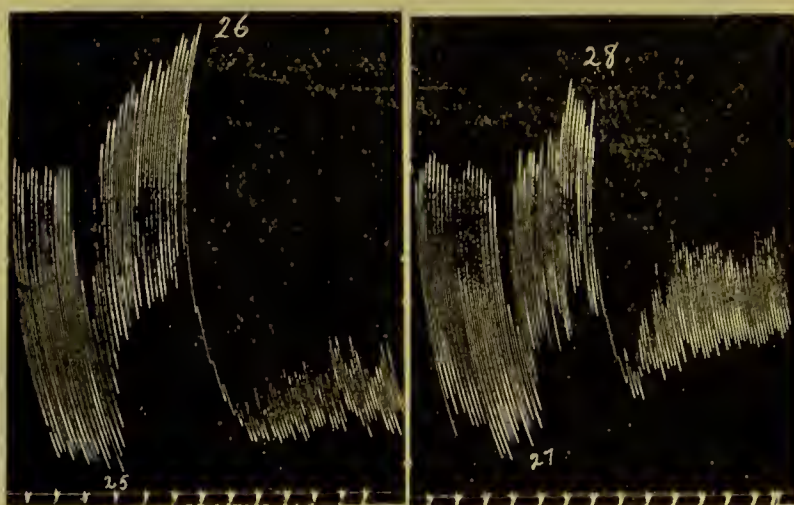


FIG. 11.

At 25, Ringer was replaced by indifferent blood (from a jugular vein) diluted with 4 volumes Ringer, and this at 26 by jugular blood to which 1:2,000,000 adrenalin was added, the mixture being then diluted with four volumes Ringer. At 27, Ringer was replaced by jugular serum, diluted with 4 volumes Ringer, and this at 28 by jugular serum to which 1:3,000,000 adrenalin was added, the mixture being then diluted with 4 volumes Ringer.

with those of blood and serum to which adrenalin had been added in known amounts. The adrenalin blood and serum before application to the segment were diluted with 4 volumes Ringer, and the adrenal vein blood and serum were similarly diluted. It will be understood, for instance, that when a blood or serum containing, say, 1:2,000,000 adrenalin is used, this is the original concentration before dilution with the 4 volumes Ringer.

At 25 (fig. 11) Ringer was replaced by jugular blood, and this at 26 by jugular blood containing 1:2,000,000 adrenalin. By comparing observation 26 with observation 20 (fig. 10) it will be obvious that 1:2,000,000 is far above the concentration of epinephrin in the adrenal vein blood. At 27 (fig. 11) Ringer was replaced by jugular serum, and this at 28 by jugular serum containing 1:3,000,000 adrenalin. If this observation be compared with observation 18 (fig. 10) it will be seen that the concentration of epinephrin in the serum of the second pocket specimen is very nearly 1:3,000,000.

In figure 12 (observation 36) it is shown that 1:7,000,000 is much below the concentration in this serum. Comparing observation 30 with observation 20 (fig. 10), it can be deduced that 1:7,000,000 is greater than the concentration in the blood of the second pocket specimen. In figure 13 it is demonstrated (observation 46) that 1:6,000,000 is much above the concentration of epinephrin in the blood of the second pocket specimen (observation 40), and that 1:10,000,000 (observation 48) is below the concentration. So far, then, we have determined that the concentration in this blood lies between 1:7,000,000 and 1:10,000,000, probably nearer the former. In figure 14 it is shown that indifferent blood containing 1:8,000,000 adrenalin (observation 66) produces almost the same effect on the intestine segment as blood of the second pocket specimen in similar dilution (observation 64).

Accordingly, in this blood the epinephrin concentration is approximately 1:8,000,000, while in the serum separated from it it is approximately 1:3,000,000. The blood of this dog was unusually rich in corpuscles, a circumstance favorable rather than otherwise for our purpose, as a given concentration of

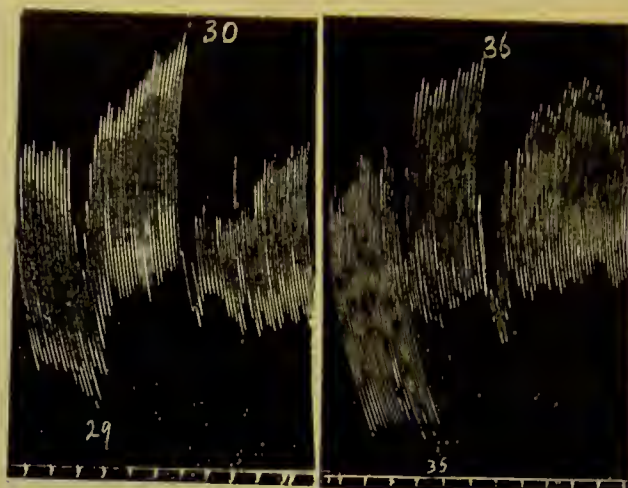


FIG. 12. RABBIT INTESTINE SEGMENT

At 29, Ringer was replaced by jugular blood, diluted with 4 volumes Ringer, and this at 30 by jugular blood to which 1:7,000,000 adrenalin was added, the mixture being then diluted with four volumes Ringer. At 35, Ringer was replaced by jugular serum, diluted with 4 volumes Ringer and this at 36 by jugular serum to which 1:7,000,000 adrenalin had been added, the mixture being then diluted with 4 volumes Ringer.

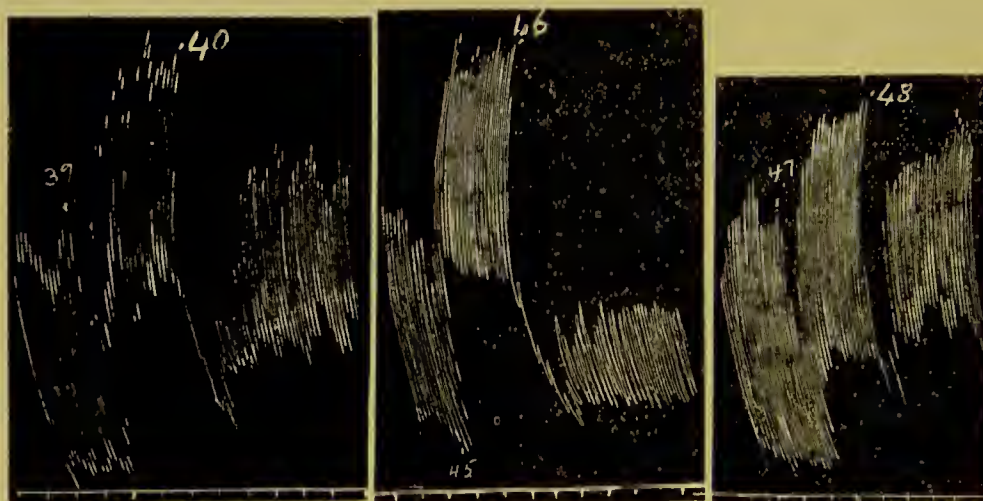


FIG. 13. RABBIT INTESTINE SEGMENT

At 39, Ringer was replaced by jugular blood, diluted with four volumes Ringer, and this at 40 by adrenal vein blood similarly diluted. At 45, Ringer was replaced by jugular blood diluted with four volumes Ringer and this at 46 by jugular blood to which 1:6,000,000 adrenalin had been added. The mixture being then diluted with 4 volumes Ringer. At 47 Ringer was replaced by jugular blood diluted with 4 volumes Ringer, and this at 48 by jugular blood to which 1:10,000,000 adrenalin had been added, the mixture being then diluted with 4 volumes Ringer.

epinephrin in the blood would correspond to a greater concentration in the serum than with a blood poorer in corpuscles. The proportion of serum in the blood of the second pocket specimen as determined by the electrical method was 36.5 per

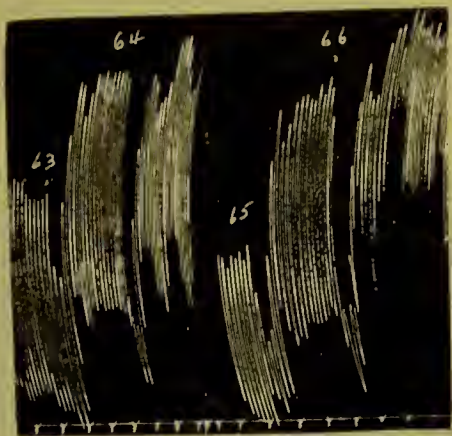


FIG. 14. RABBIT INTESTINE SEGMENT

At 63 Ringer was replaced by jugular blood diluted with 4 volumes Ringer, and this at 64 by adrenal vein blood similarly diluted. At 65 Ringer was replaced by jugular blood diluted with 4 volumes Ringer, and this at 66 by jugular blood to which 1: 8,000,000 adrenalin had been added, the mixture being then diluted with 4 volumes Ringer.

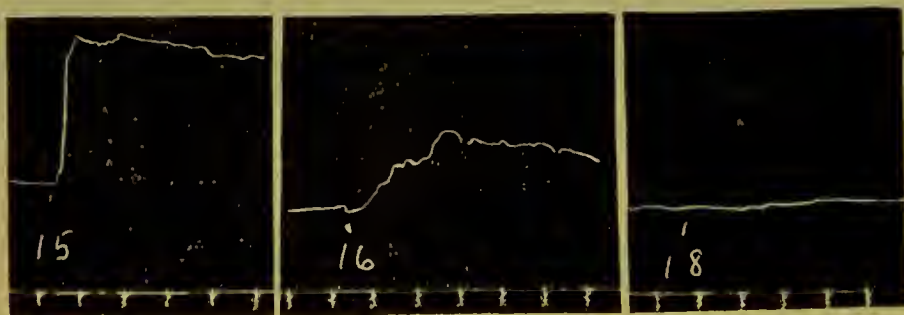


FIG. 15. EFFECT OF ADRENAL BLOOD (16), SERUM (15), AND SEDIMENT (18) ON A RABBIT UTERUS SEGMENT

All the specimens were diluted with 5 volumes Ringer

cent. By the haematocrit it was 29.5 per cent after forty minutes rotation, 33 per cent after sixty minutes, and 35 per cent after seventy minutes. The serum separated very slowly, as was also the case when the blood was centrifuged in the large centrifuge.

Taking the proportion of serum as 36 per cent, and the concentration of epinephrin in it at 1:3,000,000, the blood would contain 1:8,300,000 on the supposition that it is all in the serum.

The great deficiency of the sediment of the same specimen of adrenal vein blood in epinephrin as compared with the serum was strikingly confirmed on the rabbit uterus segment (fig. 15). Observation 15 shows the rise of tone produced when Ringer

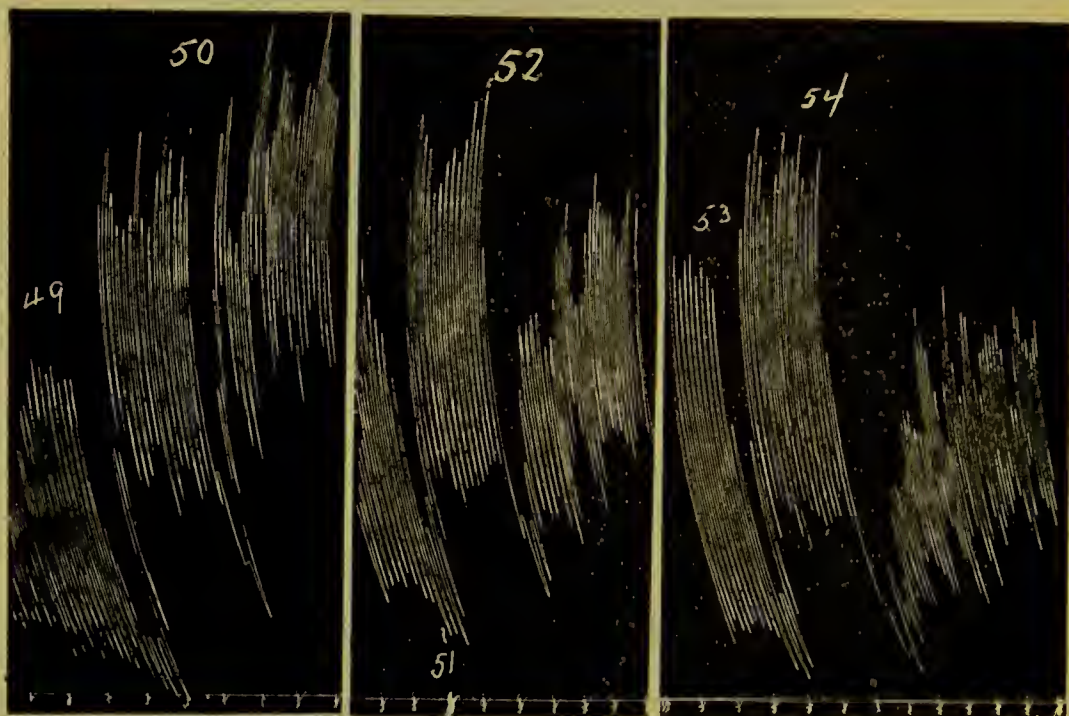


FIG. 16. RABBIT INTESTINE SEGMENT TESTS, TO SHOW INCREASE IN EPINEPHRIN CONCENTRATION IN SUCCESSIVE SAMPLES OF ADRENAL VEIN BLOOD

At 49, Ringer was replaced by jugular vein blood, and this at 50 by the first specimen of adrenal blood. At 52, the second specimen, and at 54 the third specimen of adrenal blood replaced jugular blood. All the bloods were diluted with 4 volumes Ringer.

was replaced by the serum (diluted with 5 volumes of Ringer); observation 16, the smaller increase of tone caused by the blood similarly diluted. The sediment in the same dilution (observation 18) caused practically no effect.

We may conclude, then, that within the limits of error of such an assay as is possible with intestine and uterus segments, the naturally secreted epinephrin in dog's adrenal vein blood is all

in the plasma. It follows from this that in searching for such minute concentrations of epinephrin as can exist in the blood, the chance of detection is about twice as great with serum (or plasma) as with blood. Further, the effective concentration of epinephrin which acts upon sensitive structures exposed to blood containing it is about double the concentration calculated on the volume of the entire blood. It is obvious that it is not permissible to compare serum and blood on the assumption that the epinephrin is uniformly distributed over erythrocytes and serum;

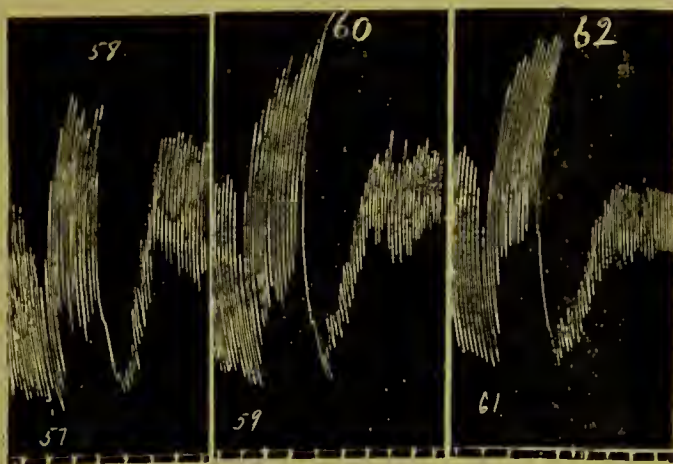


FIG. 17. EFFECT OF THE SERA FROM THE BLOODS WHOSE ACTION ON THE INTESTINE SEGMENT IS SHOWN IN FIGURE 16

At 58, serum of the first specimen of adrenal vein blood replaced serum of jugular blood. At 60, serum of the second specimen, and at 62 serum of the third specimen, replaced jugular blood serum. All the sera were diluted with 4 volumes Ringer.

to conclude, for instance, from the fact that a smaller concentration of epinephrin was found in a sample of blood than in the serum of another sample, that the rate of liberation of epinephrin had increased during the collection of the second sample.

A striking illustration of the fact that the epinephrin is in the plasma is afforded by comparison of the effects upon intestine segments of a series of samples of blood successively collected from the adrenal vein with the effects of the sera separated from them. It is known that in general the epinephrin concentration increases in the successive samples of blood, at least when the rate

of flow is progressively diminishing. The increase is far too great to be accounted for by any increase in the relative proportion of plasma to corpuscles associated with hemorrhage without change in the concentration of epinephrin in the plasma (table 1). And figures 16 and 17 show clearly that the concentration in the sera of successive samples goes on increasing. Each serum not only produces a greater inhibition of the intestine segment than the corresponding blood, but a greater inhibition than the serum of an earlier specimen.

TABLE 1

	K $\times 10^4$ AT 5°C.	PERCENTAGE OF SERUM
Jugular blood.....	19.7	35.7
Jugular serum.....	85.1	
First adrenal blood.....	20.7	36.4
First adrenal serum.....	87.1	
Second adrenal blood.....	20.6	36.5
Second adrenal serum.....	86.8	
Third adrenal blood.....	22.0	39.4
Third adrenal serum.....	85.1	

Our observations were confined to cat's and dog's blood. Whether, as in the well-known instance⁵ of dextrose, naturally secreted epinephrin or added adrenalin distributes itself differently in the blood of different species of animal there is no evidence.

SUMMARY

1. When adrenalin is added to defibrinated cat's or dog's blood it is all found in the serum within the limits of error of the methods employed for its assay (colorimetric method of Folin, Cannon and Denis; Elliott's method; rabbit intestine and uterus segments).

2. The same is true of the naturally secreted epinephrin in dog's adrenal vein blood.

⁵ Rona and Michaelis: *Biochem. Zeitsch.* 1909, xvi, 60; xviii, 375, 514; 1910, xxx, 99, etc.

THE INFLUENCE OF ASPHYXIA UPON THE RATE OF LIBERATION OF EPINEPHRIN FROM THE ADRENALS¹

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The hyperglycaemia and glycosuria associated with asphyxia have been explained by some writers as due to stimulation of the adrenals to increased liberation of epinephrin. This, however, is a hypothesis unsupported by any conclusive evidence showing that in asphyxia the rate at which epinephrin is discharged is, as a matter of fact, increased. Since it has been proved that the liberation of epinephrin from the adrenals is under the control of nerves, it would seem probable that asphyxia, which causes excitation of so many nervous centers, might excite the central mechanism on which the epinephrin secretion depends. We endeavored to put the question to the test by collecting adrenal blood in a vena cava pocket, and then by releasing the pocket permitting it to elicit the reactions appropriate to epinephrin on the blood pressure. In cases in which the pupil was not too greatly dilated by the asphyxia the (denervated) eye reactions, after excision of the superior cervical ganglion according to the procedure of Meltzer, were also employed. The results were negative (1). No clear difference could be made out in the magnitude of the reactions, in cats, when adrenal blood was collected in the pocket for equal times with and without asphyxia. As the epinephrin is, of course, greatly diluted before it reaches

¹ A preliminary account of the work was given at the joint meeting of the Federation of American Societies for Experimental Biology, December 28, 1916. A note was published in the Proceedings of the Society for Experimental Biology and Medicine, January 17, 1917.

the sensitive structures concerned in the blood pressure and eye reactions, we have repeated the observations with unmixed adrenal blood withdrawn from the cava pocket by a cannula and tested upon rabbit intestine and uterus segments according to the method introduced by one of us (2). Specimens of adrenal blood were collected for accurately measured periods of time, with free and with obstructed respiration. Since when the adrenal blood flow is diminishing in successive samples, the concentration of epinephrin goes on increasing, the asphyxial and non-asphyxial periods did not follow each other in any definite order, so that an increase of concentration due merely to the diminution in the blood flow might not simulate an increase due to asphyxia. Special weight was also given to observations in which the successive samples, with and without asphyxia, were collected with unchanged rate of blood flow. Experiment 1 is an example of such experiments.

Experiment 1. Condensed protocol. Cat (male). 3.41 kgm., weight. Urethane. A sample of indifferent blood was obtained from the jugular vein. Then a cava pocket ("short" pocket) was made, the renal, coeliac, mesenteric arteries and abdominal aorta below the renals being tied. The following adrenal blood samples were then collected.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	minutes	seconds	grams	
1	2.0		50	2.2	No asphyxia
2	2.9	1	15	2.3	Asphyxia
3	3.1	1	20	2.4	Asphyxia
4	3.3	1	45	1.9	Without asphyxia
5	4.0	2		2.0	Without asphyxia
6	3.2	2		1.6	Asphyxia
7	2.0	1		2.0	Without asphyxia
8	6.5	4	25	1.5	Without asphyxia

Blood was now obtained from the abdominal aorta. Combined weight of adrenals, 0.550 gram.

In figure 1, it will be seen that the sixth adrenal specimen (collected during asphyxia) and the fifth specimen (collected

without asphyxia) caused almost the same amount of inhibition of the intestine. The same thing is seen in figure 2, where the two specimens were compared in a different dilution. If the sixth specimen has a slightly greater effect this is certainly no more than would be associated with the somewhat smaller rate of blood flow when the sixth sample was being collected. If asphyxia is capable of stimulating the adrenal to increased

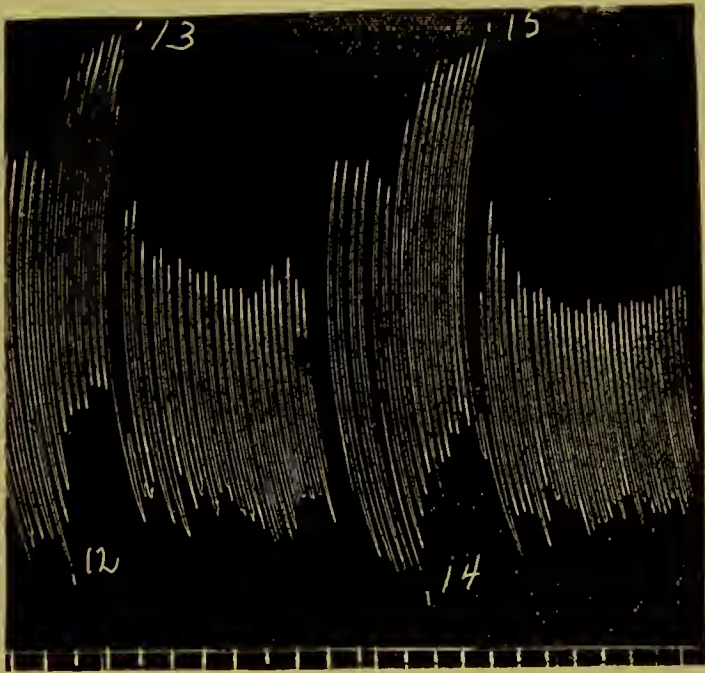


FIG. 1. INTESTINE TRACINGS. BLOOD FROM CAT ANESTHETIZED WITH URETHANE

At 12, Ringer was replaced by jugular blood, and this at 13 by the fifth adrenal blood specimen, collected without asphyxia. At 14, Ringer was replaced by jugular blood, and this at 15 by the sixth adrenal blood specimen, collected during asphyxia. The bloods were diluted with six volumes of Ringer. As in all the tracings, the time is marked in half minutes. (Reduced to two-thirds.)

secretion of epinephrin, a marked preponderance of epinephrin concentration ought to have been seen in the sixth specimen. In figure 3, the first adrenal specimen (collected without asphyxia) is seen to produce at least as great an effect as the third specimen (collected during asphyxia). The blood flows being practically equal during the collection of these two specimens, it can be assumed that the concentrations of epinephrin in them would

have been equal had they been both collected during free respiration. The obvious conclusion is that asphyxia produced either no effect whatever on the rate of output of epinephrin; or so small an effect, that this was below the threshold of detectability with the intestine segments employed. This conclusion was confirmed on rabbit uterus segments (fig. 4). The fifth adrenal specimen caused quite as great an increase of tone as the third,

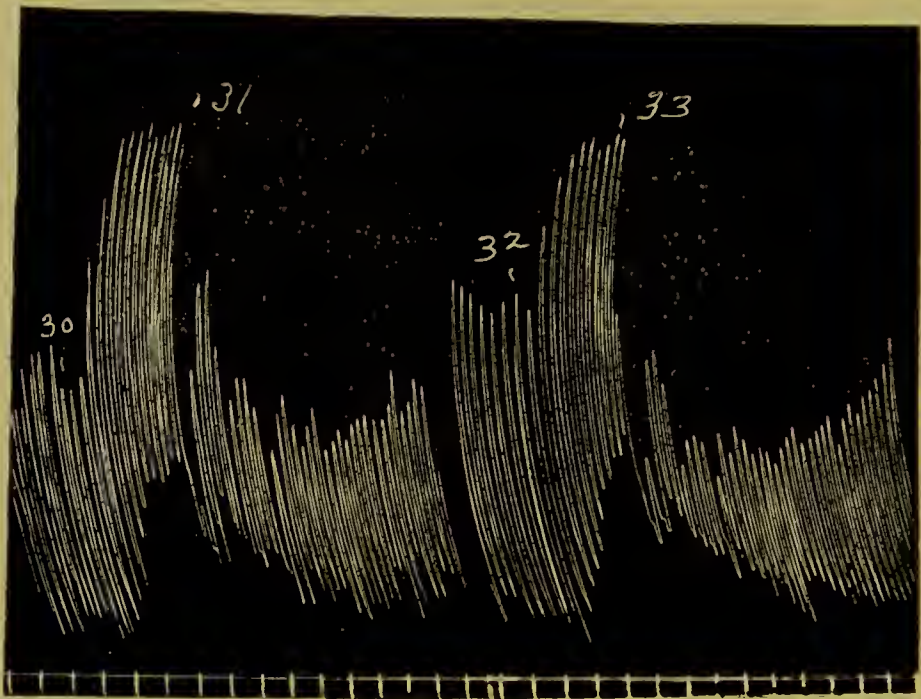


FIG. 2. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURE 1

At 30 Ringer was replaced by jugular blood, and this at 31 by the sixth adrenal specimen (asphyxia). At 32 Ringer was replaced by jugular blood, and this at 33 by the fifth adrenal specimen (without asphyxia). Bloods diluted with eight volumes Ringer. (Reduced to two-thirds.)

and the sixth specimen showed no definite preponderance over the fifth. An increase in adrenalin concentration from 1:3,000,000 (fig. 4, observation 40) to 1:2,000,000 (observation 41) could have been easily detected by the uterus segment, and no doubt a much smaller difference.

Other experiments of this type on cats yielded a similar result. No clear evidence was obtained in any of them from a comparison

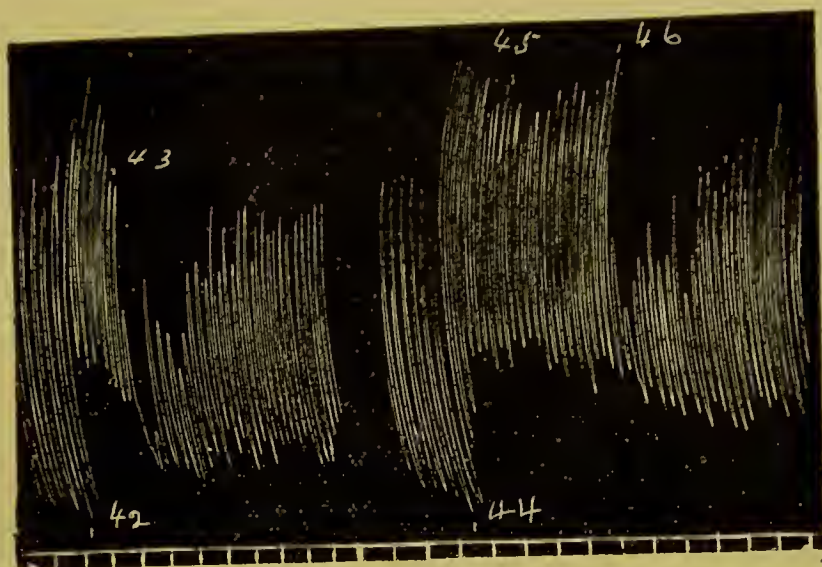


FIG. 3. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURES 1 AND 2

At 42 Ringer was replaced by jugular blood, and this at 43 by the first adrenal blood specimen (without asphyxia). At 44 Ringer was replaced by jugular blood. At 45 some more jugular blood was added, which was replaced at 46 by the third adrenal specimen (asphyxia). Bloods diluted with eight volumes Ringer. (Reduced to two-thirds.)

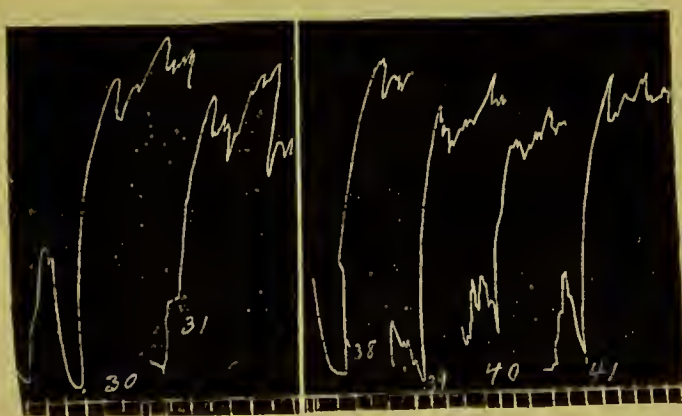


FIG. 4. UTERUS TRACINGS WITH BLOOD FROM CAT USED FOR FIGURES 1 TO 3

At 30 Ringer was replaced by the fifth adrenal blood specimen (without asphyxia); at 31 by the sixth adrenal specimen (asphyxia). Both bloods were diluted with twenty volumes Ringer. At 38 Ringer was replaced by the fifth adrenal specimen; at 39 by the third adrenal specimen (asphyxia). Both bloods diluted with nine volumes Ringer. At 40 adrenalin in carotid blood (1:3,000,000) and at 41 adrenalin in carotid blood (1:2,000,000) replaced Ringer. Both adrenalin bloods, after being made up to the concentrations mentioned, were diluted with nine volumes Ringer before application to the segment. The weight was increased between observations 31 and 38. (Reduced to one-half.)

of adrenal bloods collected during and without asphyxia that the rate of epinephrin output was sensibly increased by asphyxia. In one of these experiments on a cat anesthetized with urethane, adrenal blood collected during asphyxia was compared with adrenal blood collected during stimulation of sensory nerves (sciatic) (3), and both specimens with adrenal blood collected during free respiration and without sensory stimulation. No unequivocal difference in the rate of liberation of epinephrin in the different samples could be made out.

In the next experiment a dog was employed, to insure such a large flow of blood that the dead space in the cannula and cava pocket would be very quickly washed out between successive samples. In the cats, the "overlapping" of the asphyxial and non-asphyxial specimens was reduced to a minimum by beginning the asphyxia a little before the completion of collection of the preceding non-asphyxial specimen, and stopping it a little before the end of the collection of the asphyxial specimen. From the very large blood flows into the pocket in experiment 2, it is possible that some small vein going to the pocket was left untied, although no evidence of this was found at the end of the experiment. It is easier in the cat than in the dog to be certain that nothing has escaped ligation. However, in connection with the relatively large blood flow commonly observed in these experiments, both in cats and dogs, it must be noted that usually the blood pressure was quite high during a considerable part of the experiment, since so many arteries (renal, coeliac, mesenteric and abdominal aorta) were tied off. Whether a small leak existed or not in this dog makes no difference to the result of the experiment, since it would affect all the samples proportionally.

Experiment 2. Condensed protocol. Dog (female). Weight, 7.5 kgm. Ether. Cava pocket made (renal, coeliac, mesenteric and both iliac arteries tied). Cannulae inserted into each iliac vein. Indifferent blood obtained from jugular vein. The following specimens of adrenal blood were then collected from the cava pocket:

The first four specimens were collected through the right iliac cannula, and the last three through the left. While clipping off the right

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	cc.	minutes	seconds	cc.	
1	8.05		20	24.0	Asphyxia begun
2	37.1	2		18.5	Asphyxia
3	30.5	2		15.2	Without asphyxia
4	23.5	1	47	13.4	Without asphyxia
5	35.0	2	5	16.6	Asphyxia
6	23.0	2	20	10.0	Without asphyxia
7	14.3	2	15	6.35	Without asphyxia

and releasing the left the pocket filled up, and the amount of blood collected in the fifth specimen is greater than it should have been. Thus the greater flow for the fifth specimen given in the table is only apparent. The epinephrin concentration would of course not be affected by this as it remained occluded at the upper end. Combined weight of adrenals, 0.96 gram.

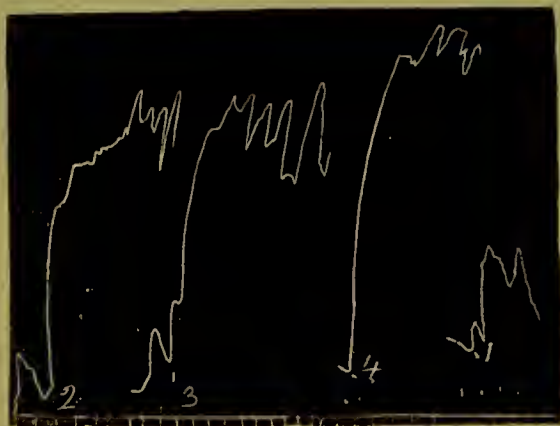


FIG. 5. UTERUS TRACINGS

At 1 Ringer was replaced by carotid blood from a dog; at 2 by the second adrenal blood specimen from the dog, collected during asphyxia; at 3 by the third adrenal blood specimen, collected without asphyxia; at 4 by the fourth adrenal specimen, collected without asphyxia. All bloods diluted with four volumes Ringer. (Reduced to one-half.)

In figure 5, some of the uterus tracings from experiment 2 are reproduced. Observations 2 and 3 show that the third adrenal sample (collected without asphyxia) is at any rate as rich in epinephrin as the second (collected during asphyxia). Intestine

segment tracings, not reproduced, demonstrated that the third specimen contained, indeed, somewhat more epinephrin than the second, which agrees with the slightly greater blood flow during collection of the second specimen. The progressive increase in concentration in successive samples was well shown on the uterus segments, for the adrenal specimens from the fifth to the seventh, despite the fact that the sixth and seventh samples were collected without asphyxia and might therefore have been expected to possess a smaller content of epinephrin than the fifth sample, had asphyxia been capable of increasing the output to an extent detectable by these methods.

It might be objected that under the experimental conditions (anesthesia, trauma, etc.) the output of epinephrin was so stimulated that it was already at or near the possible maximum in the periods of free respiration. In this case asphyxia could not cause a demonstrable increase. Although it is easy to show that the rate of liberation under the same conditions can be readily increased through the secretory nerves, namely, by electrical stimulation of the splanchnic, some experiments were made in which the use of a chemical anesthetic was avoided, naturally by the substitution of methods which rendered the animal completely insensitive. No difference in the result was found.

Experiment 3. Condensed protocol. Dog (male). Weight, 11.05 kgm. Rendered insensitive by destruction of the cerebral cortex with some of the underlying centrum ovale. Under ether, trephined and curetted away the cerebral cortex. Tracheal and jugular cannulae inserted and specimen of jugular blood obtained. "Short" cava pocket made, renal, coeliac and mesenteric arteries and abdominal aorta being tied. Started artificial respiration, although the animal was breathing spontaneously, and collected the following adrenal blood samples:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	minutes	seconds	grams	
1	7.0	1	45	4.0	Without asphyxia
2	7.7	2	35	3.0	Without asphyxia
3	8.5	4		2.1	During asphyxia
4	8.8	5	40	1.5	During asphyxia
5	4.5	6		0.75	Without asphyxia

A sixth specimen was collected during asphyxia, but partial clotting in the cannula prevented proper estimation of the rate of flow.

Specimens of the uterus tracings from experiment 3 are reproduced in figure 6. A regular increase in the effect is observed from the second to the sixth adrenal specimen, without any apparent relation to the presence or absence of asphyxia. The second specimen (observation 28) caused a smaller increase

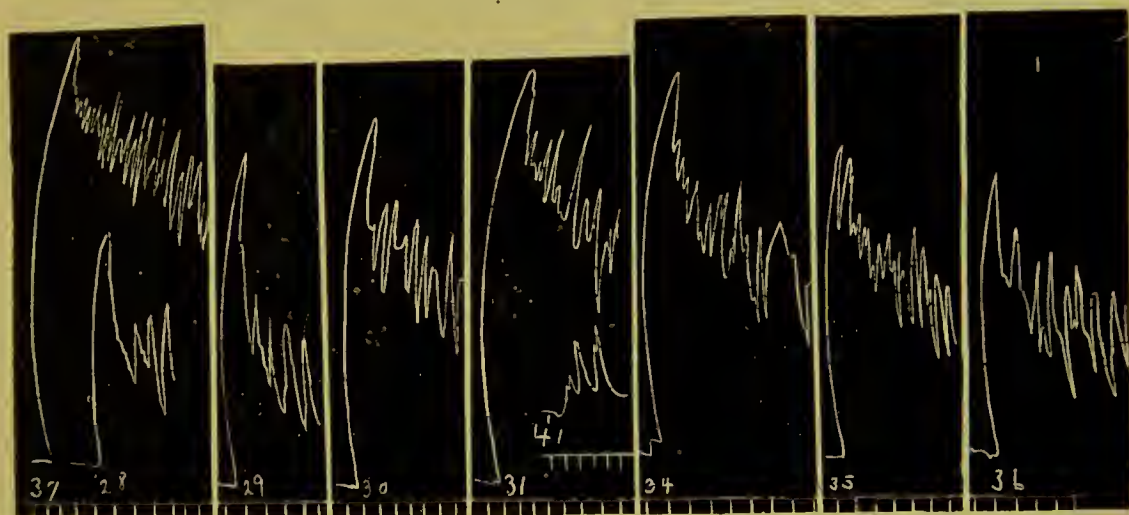


FIG. 6. UTERUS TRACINGS. BLOOD FROM DOG WITH CEREBRAL HEMISPHERES DESTROYED

At 28 Ringer was replaced by the second adrenal specimen (without asphyxia); at 29 by the third adrenal specimen (asphyxia); at 30 by the fourth adrenal specimen (asphyxia); at 31 by the fifth adrenal specimen (without asphyxia); at 37 by the sixth adrenal specimen (asphyxia); at 41 by jugular vein blood. All bloods diluted with fifteen volumes Ringer. At 34 adrenalin in jugular blood (1:2,000,000); at 35 adrenalin in jugular blood (1:3,000,000); at 36 adrenalin in jugular blood (1:4,000,000) replaced Ringer. The adrenalin bloods, after being made up to the concentrations mentioned, were diluted with 15 volumes Ringer before application to the segment. (Reduced to one-half.)

of tone in the segment than the third (observation 29). But this was not due to its having been collected during asphyxia, for the fifth specimen (observation 31), collected without asphyxia, produced a decidedly greater effect than the fourth (observation 30), collected during asphyxia. The third and fourth specimens were both collected during asphyxia; but the fourth caused the greater effect upon the uterus, particularly as regards

the persistence of the increased tone. The somewhat greater concentration of epinephrin in the fourth specimen, as compared with the third, cannot be connected with any stimulation of the epinephrin secretion by asphyxia, since this would equally be present during collection of the third specimen. It is undoubtedly due to the fact that the rate of flow when the fourth specimen was being obtained was somewhat less than when the third specimen was being obtained. The rate of liberation of epinephrin per minute being approximately constant at this time, a diminution in the rate of blood flow was necessarily associated with a corresponding increase in the epinephrin concentration. An increase in concentration from 1:4,000,000 to 1:3,000,000, or from 1:3,000,000 to 1:2,000,000 (observations 34 to 36) could easily be detected by the uterus segment, and assuredly much smaller changes in concentration.

Elliott (4) states that after brain mutilations in cats the epinephrin store of the adrenals is markedly diminished through the nerves coming to the semilunar ganglion from the sympathetic. He considers that this is due to irritation of a secretory nerve path caused by the brain lesion leading to increased discharge of epinephrin. However, he made no experiments to show that the rate of liberation is, as a matter of fact, increased. Assays of adrenal blood specimens from the dog studied in experiment 3 showed, indeed, that the output of epinephrin per kilogram of body weight per minute was less than that usually found in anesthetized dogs with intact central nervous system. The lack of the stimulating action on the secretory mechanism attributed by various writers to anesthetics might seem to afford an explanation. There is no real proof, however, that anesthetics possess such an action. In any case, the moderate rate of output of epinephrin with free respiration ought to have supplied the most favorable condition for bringing out an asphyxial increase, if any decided increase could be produced by asphyxia.

In the next experiment (experiment 4), mutilation of the brain and chemical anesthesia were both avoided by rendering the animal insensitive through increase of intracranial pressure.

Experiment 4. Condensed protocol. Dog (female). Weight, 10.4 kgm. Under ether, trephined; inserted rubber bag (condom) through trephine hole; brought up the pressure to 250 mm. of mercury, and kept the pressure between 200 and 250 mm. through the whole time of collection of the adrenal bloods. Ether was discontinued as soon as the pressure was begun, and artificial respiration was started. A sample of jugular blood was obtained. Then a "short" cava pocket was made, the renal, coeliac and mesenteric arteries and abdominal aorta being tied. The following specimens of adrenal blood were collected:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	minutes	seconds	grams	
1	6.3	1	10	6.0	Without asphyxia
2	6.8	4	15	1.6	With asphyxia
3	4.5	3	20	1.4	Without asphyxia
4	5.7	3	40	1.6	Without asphyxia
5	6.8	4		1.7	During asphyxia
6	9.0	5	30	1.7	During asphyxia
7	7.7	3		2.6	Without asphyxia
8	13.8	5	20	2.6	Without asphyxia
9	9.0	5		1.8	With greater degree of asphyxia than before
10	6.4	2	40	2.5	Without asphyxia

Combined weight of adrenals, 1.46 grams. Clot was removed from the cannula between collection of the fourth and fifth samples, and again between the ninth and tenth samples.

In figures 7 and 8 are reproduced some of the intestine segment tracings from experiment 4. Figure 7 shows that the third adrenal specimen (obtained without asphyxia) had a somewhat greater inhibitory power than the second specimen (collected during asphyxia), corresponding to the somewhat slower flow of the third specimen. The difference in the flows, however, was so slight that asphyxial stimulation of the secretion ought to have easily produced an excess of epinephrin in the second specimen had any detectable stimulation existed. The concentration of epinephrin in the third specimen was about 1:3,000,000 (fig. 8, observations 40 and 44), and much greater than 1:5,000,000 (observation 42). Similar comparisons of the

other samples collected with free respiration and during asphyxia yielded the same result. In no case was any difference found which could be attributed to asphyxia. This was not because the concentrations were already maximal, although owing to the relatively small blood flow they were fairly high for a dog.

The objection might be offered that the high intracranial pressure had rendered the central mechanism concerned in the

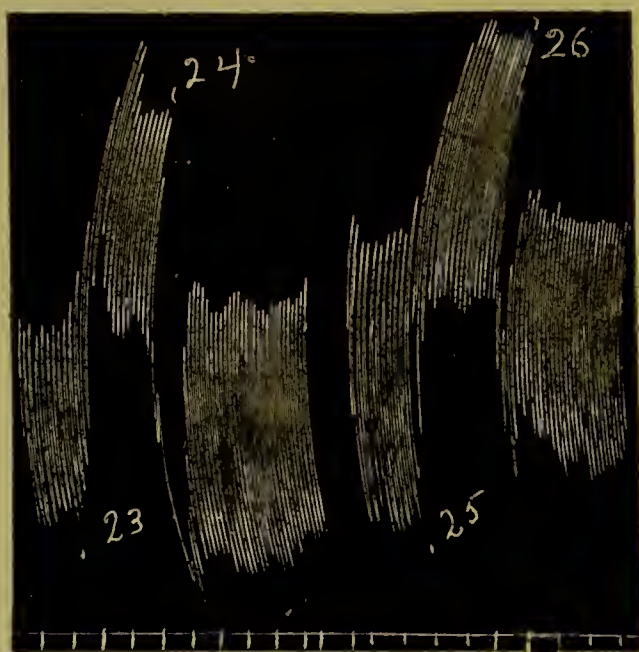


FIG. 7. INTESTINE TRACINGS. BLOOD FROM DOG RENDERED INSENSITIVE BY INCREASED INTRACRANIAL PRESSURE (EXPERIMENT 4)

At 23 Ringer was replaced by jugular blood, and this at 24 by the third adrenal specimen (collected without asphyxia). At 25 Ringer was replaced by jugular blood, and this at 26 by the second adrenal specimen (asphyxia). Bloods diluted with two volumes Ringer. (Reduced to two-thirds.)

liberation of epinephrin anemic, supposing it to be situated within the skull, and that it was therefore incapable of responding to the asphyxial blood.² We have shown, however, that

² It was, indeed, with the object of testing this idea that the high intracranial pressure was maintained throughout the experiment. Two of the adrenal blood samples, the fifth and the eighth, had a decidedly smaller concentration of epinephrin, as tested by rabbit intestine segments, than samples obtained earlier, as well as later in the series. We have never observed this phenomenon except

(in the cat) a central mechanism for epinephrin secretion exists in the upper part of the thoracic cord (5); and there is no apparent reason why this should not have been stimulated by asphyxia. Further, it must be distinctly pointed out that even if it were clearly proved that a "center" exists in the bulb or higher, the integrity of which is essential to the reduction of the epi-

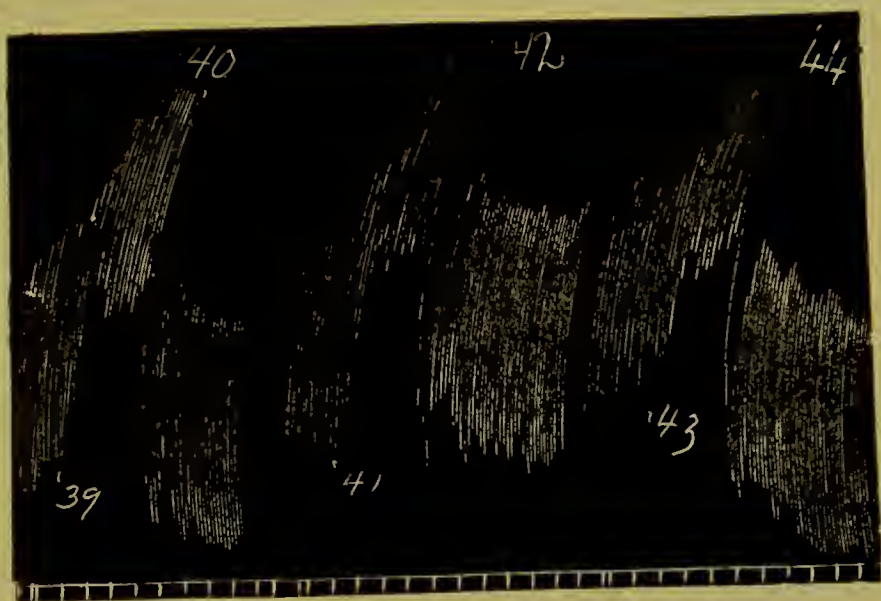


FIG. 8. INTESTINE TRACINGS. BLOOD FROM THE SAME DOG USED FOR FIGURE 7

At 39 Ringer was replaced by jugular blood and this at 40 by the third adrenal blood specimen (without asphyxia). At 41 Ringer was replaced by jugular blood, and this at 42 by adrenalin (1:5,000,000) in jugular blood. At 43 Ringer was replaced by jugular blood and this at 44 by adrenalin (1:3,000,000) in jugular blood. The bloods were diluted with two volumes Ringer, the adrenalin bloods being first made up in undiluted blood to the concentrations mentioned, and the mixture then diluted (with two volumes Ringer). (Reduced to two-thirds.)

nephrin store of the adrenals under various conditions (Elliott (4)), this is no direct proof that such a center controls the rate of

in two dogs with increased intraerianial pressure. In the cats with increased intraerianial pressure it was not seen. In any case, in the experiment under discussion the deficiency of epinephrin in these two samples affords no evidence that asphyxia stimulates the secretion of epinephrin. For one of the specimens (the fifth) was collected during asphyxia and the other (the eighth) with free respiration. It is conceivable, of course, that an intraerianial "centre" already crippled by anaemia, might have its paralysis completed by a period of asphyxia.

liberation of epinephrin into the blood. For an increased output of epinephrin is not the only way in which a diminution of the epinephrin store in the adrenals could be caused. Nevertheless, in experiment 5 an attempt was made to take account of this objection by lowering the intracranial pressure as soon as the animal became insensitive. The result of Experiment 5, however, differed in no essential way from that of Experiment 4.

Experiment 5. Condensed protocol. Dog (male). Weight, 9.25 kgm. Rendered insensitive by increased intracranial pressure. Under ether, trephined and inserted rubber bag. Got up pressure to 250 mm. of mercury. Discontinued ether. Decreased the pressure as the blood pressure fell. Started artificial respiration. Obtained specimen of jugular blood. Made short cava pocket, tying renal, coeliac and mesenteric arteries and abdominal aorta. Collected the following specimens of adrenal blood:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	minutes	seconds	grams	
1	14.3	1		14.3	Without asphyxia
2	8.7		45	11.6	Without asphyxia
3	8.8	1	50	5.0	During asphyxia
4	6.7	1	40	4.2	During asphyxia
5	14.9	2	15	6.6	Without asphyxia
6	12.4	2	30	5.0	Without asphyxia
7	20.7	2	55	6.8	During asphyxia
8	10.3	1	30	6.8	Without asphyxia

Another specimen of jugular blood was now obtained. All the bloods were centrifuged, and the sera tested on rabbit intestine and uterus segments. Combined weight of adrenals, 1.32 grams.

Specimens of intestine segment tracings from experiment 5 are reproduced in figures 9 and 10. In figure 9, it is shown that serum of the sixth adrenal specimen (observation 21) caused practically the same inhibition as serum of the seventh specimen (observation 23), although the seventh was collected during asphyxia, and the sixth with free respiration. Adrenalin assays (samples of the tracings are reproduced in fig. 10), proved that

the sera of the sixth and seventh specimens contained much more than 1:8,000,000 epinephrin (observation 29), and as nearly as possible 1:5,000,000 (observation 27), corresponding to an output of less than 0.0001 mgm. per kilo of body weight per minute. This is below rather than above the average output for dogs anesthetized with ether, and the concentration is considerably below the average for the sera, as estimated in this way.

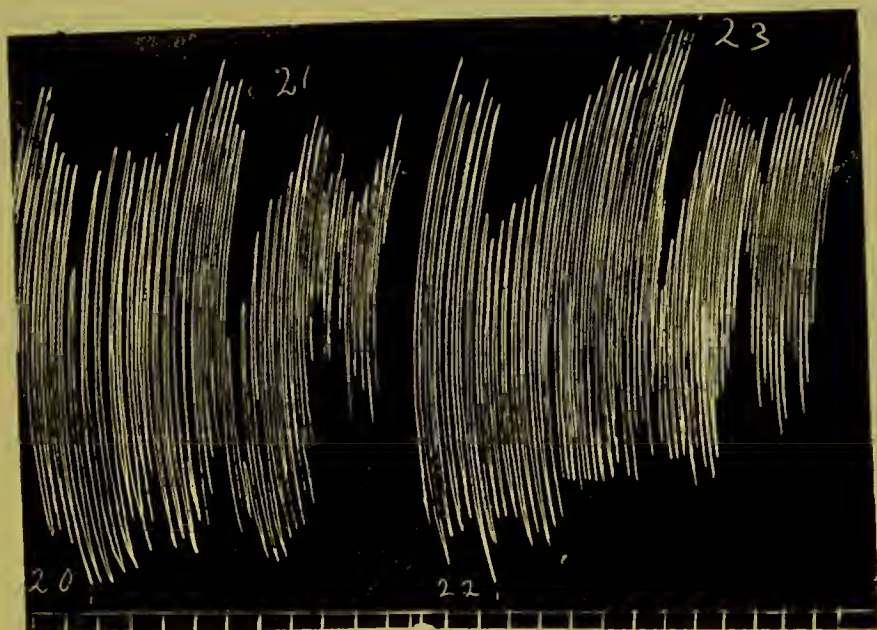


FIG. 9. INTESTINE TRACINGS. BLOOD SERUM FROM DOG RENDERED INSENSITIVE BY INCREASED INTRACRANIAL PRESSURE (EXPERIMENT 5)

At 20 Ringer was replaced by jugular blood serum, and this at 21 by serum of the sixth adrenal specimen (without asphyxia). At 22 Ringer was replaced by jugular serum, and this at 23 by the serum of the seventh adrenal specimen (asphyxia). All the sera were diluted with two volumes Ringer. (Reduced to two-thirds.)

The concentration of epinephrin in those sera is much below the possible maximum. Therefore, it ought to have been easy to detect an increase due to asphyxia, had asphyxia been capable of producing a great and abrupt augmentation in the output.

Some of the uterus tracings are reproduced in figure 11. The fifth adrenal serum, collected without asphyxia, caused a somewhat greater increase of tone than the fourth, collected during

asphyxia, despite the fact that the blood flow for the fifth was rather greater than for the fourth specimen; and that the concentration in the fifth specimen might therefore have been expected to be a little less than in the fourth. The difference between the two specimens was confirmed by observations 43 and 45, made with a greater degree of dilution. With a smaller dilution, the increase of tone was the same for the two speci-

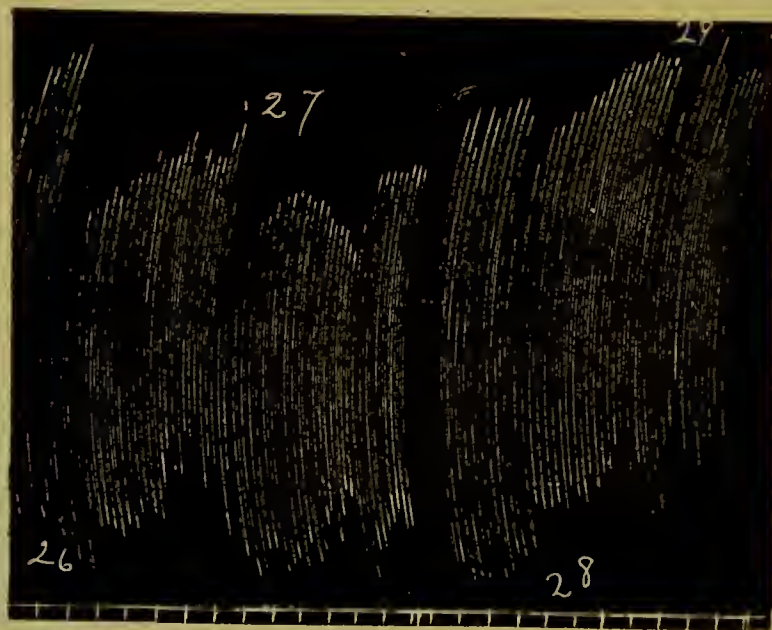


FIG. 10. INTESTINE TRACINGS. SERA FROM SAME DOG USED FOR FIGURE 9

At 26 Ringer was replaced by jugular serum, and this at 27 by adrenalin (1:5,000,000) in jugular serum. At 28 Ringer was replaced by jugular serum and this at 29 by adrenalin (1:8,000,000) in jugular serum. The adrenalin sera, after being made up to the concentrations mentioned, were diluted with two volumes Ringer before application to the segment. (Reduced to two-thirds.)

mens (observations 46 and 47), this being approximately the maximal increase of which the segment was capable in response to the combined serum and epinephrin effects of these sera. Observations 32 and 33 (on another uterus segment) gave practically the same effect for the sera of the sixth and seventh adrenal specimens, when the persistence of the increase of tone is taken into account, the uterus response being almost maximal. In a greater dilution, however, a difference was brought out

(observations 35 and 36) in favor of the seventh specimen, collected during asphyxia. The intestine tracings showed that any difference which existed between these two specimens must have been slight. It must be remarked here that in comparing uterus tracings the absolute difference in height has not the

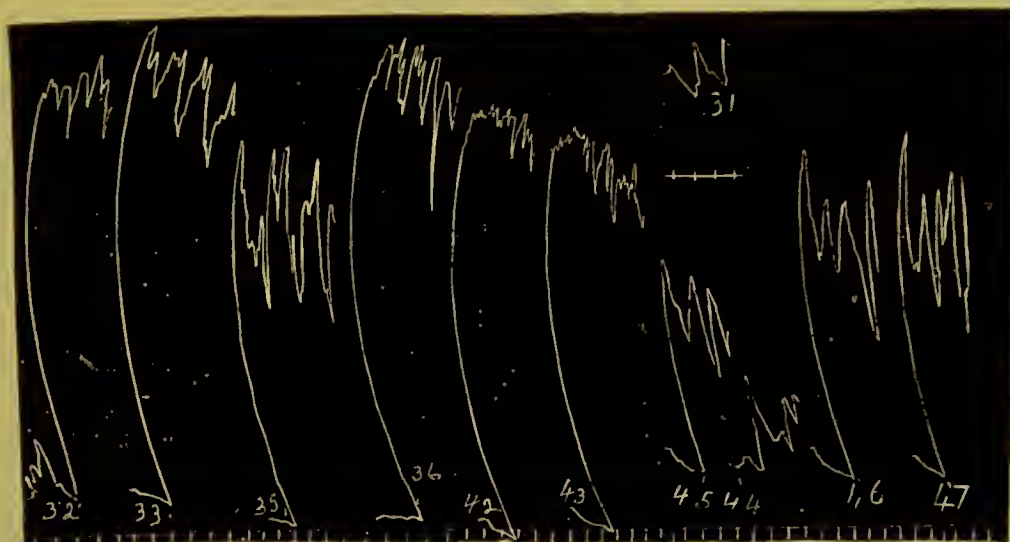


FIG. 11. UTERUS TRACINGS. SERA OF SAME DOG USED FOR FIGURES 9 AND 10

At 31 Ringer was replaced by jugular serum; at 32 by serum of sixth adrenal specimen (collected without asphyxia); at 33 by serum of seventh adrenal specimen (asphyxia). The three sera were diluted with four volumes Ringer. At 35 Ringer was replaced by serum of the sixth adrenal blood specimen; at 36 by serum of the seventh specimen, in each case diluted with six volumes Ringer. At 42 Ringer was replaced by serum of the fifth adrenal specimen (without asphyxia); at 43 by serum of the fourth adrenal specimen (asphyxia). The sera were diluted with four volumes Ringer. At 44 Ringer was replaced by serum of the fourth adrenal specimen; at 45 by serum of the fifth specimen, in each case diluted with six volumes Ringer. At 46 Ringer was replaced by serum of the fourth adrenal specimen; at 47 by serum of the fifth specimen, each diluted with three volumes Ringer. Observations 31 to 36, inclusive, were made on one uterus segment; observations 42 and 43 on another segment of the same uterus; observations 44 to 47 on a segment of another uterus. (Reduced to one-half.)

same quantitative value as the difference in the amount of inhibition of the intestine segments. All that can be deduced from observations 35 and 36 is that the combined serum and epinephrin effect of the seventh specimen is greater than that of the sixth. No estimate can be formed from these observations

as to the amount of the difference. A sensitive uterus segment practically always gives a somewhat larger effect for a later than for an earlier specimen in the absence of asphyxia, and the asphyxial periods in this experiment do not seem to have sensibly modified this progression. The blood flows did not vary much from the fifth to the eighth specimens.

A similar experiment to experiment 4 was performed on a cat (experiment 6), and with a similar negative result as regards any influence of asphyxia upon the epinephrin output.

Experiment 6. Condensed protocol. Cat (female). Weight, 2.035 kgm. Rendered insensitive by increased intracranial pressure.

10.35 a.m. Under ether, trephined, and inserted pressure bag. Got up pressure to 250 mm., and discontinued ether.

10.45 a.m. Inserted tracheal and jugular cannulae. Obtained jugular blood.

11.05 a.m. Short cava pocket made, the renal, coeliac and mesenteric arteries and abdominal aorta being tied.

11.10 a.m. Started artificial respiration, although the cat was breathing well spontaneously.

11.15 a.m. Pressure 250 mm. Eye reflexes just gone. Some voluntary respirations. Pulse, 175. Lowered pressure to 170 mm.

11.17 a.m. Lowered pressure to 130 mm. Gasping respirations and tongue movements.

11.18 a.m. Pressure raised to 230 mm.

11.20 a.m. Started collection from cava pocket.

11.21 a.m. to 11.24 a.m. Gasping respirations. Pulse, 156. Pressure raised to 260 mm. and maintained above 200 mm. during collection of the following adrenal blood specimens.

NUMBER OF ADREAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	minutes	seconds	grams	
1	2.5	1		2.5	Without asphyxia
2	4.5	2		2.2	Without asphyxia
3	3.6	2	45	1.3	Without asphyxia
4	2.5	3	30	0.7	During asphyxia
5	1.7	4		0.4	Without asphyxia
6	2.0	6	30	0.3	Without asphyxia

Spontaneous respirations were present during collection of the second and third specimens. No eye reflexes were present during collection of the adrenal samples. Blood was obtained at the end from the abdominal aorta. Combined weight of adrenals, 0.338 gram.

Some of the intestine tracings are reproduced in figures 12 and 13. The third specimen (observation 17), collected without

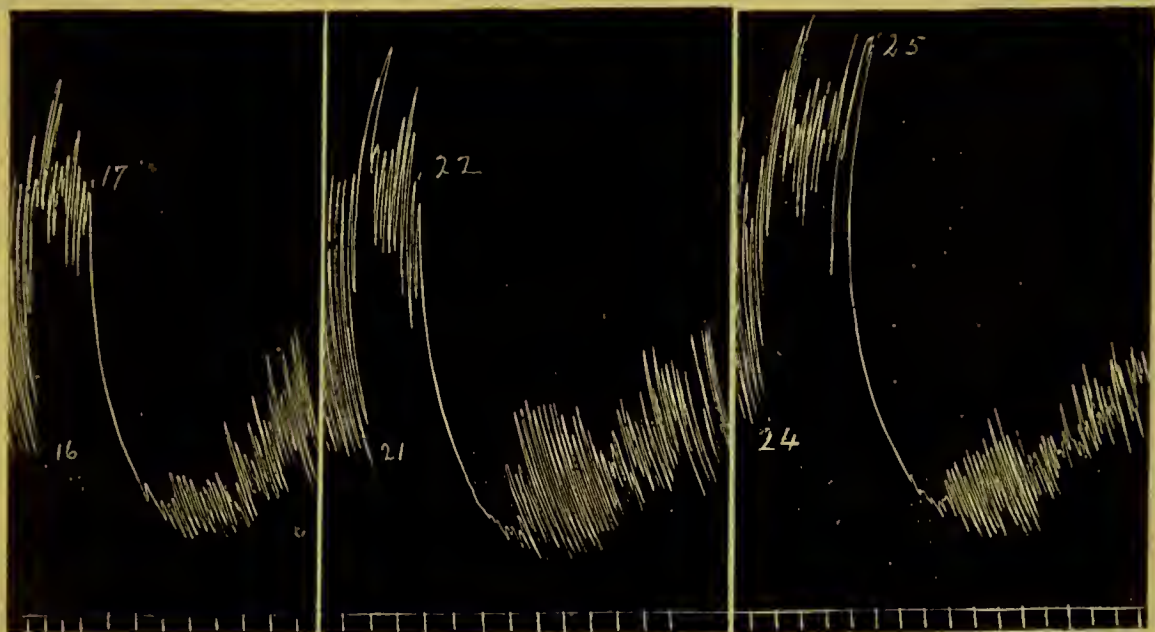


FIG. 12. INTESTINE TRACINGS. BLOOD FROM CAT RENDERED INSENSITIVE BY INCREASED INTRACRANIAL PRESSURE

At 16 Ringer was replaced by jugular blood and this at 17 by the third adrenal specimen (collected without asphyxia). At 21 Ringer was replaced by jugular blood and this at 22 by the fourth adrenal specimen (asphyxia). At 24 Ringer was replaced by jugular blood and this at 25 by the fifth adrenal specimen (without asphyxia). Bloods diluted with eight volumes Ringer. (Reduced to one-half.)

asphyxia, certainly produces a somewhat smaller effect upon the intestine segment (the inhibition is sooner recovered from) than the fourth specimen (observation 22), collected during asphyxia. But this is clearly associated with the greater flow during collection of the third specimen, and the fourth adrenal specimen is no richer in epinephrin than the fifth specimen (observation 25), collected without asphyxia. The concentration in the

fourth specimen is really less than in the fifth. With a greater degree of dilution (fig. 13), the greater effect of the fifth specimen than of the fourth in inhibiting the intestine segment became more evident. The slightly inferior inhibitory power of the third specimen as compared with the fourth is thus seen to have no demonstrable relation to the presence or absence of asphyxia. The adrenalin assay showed that the fifth specimen

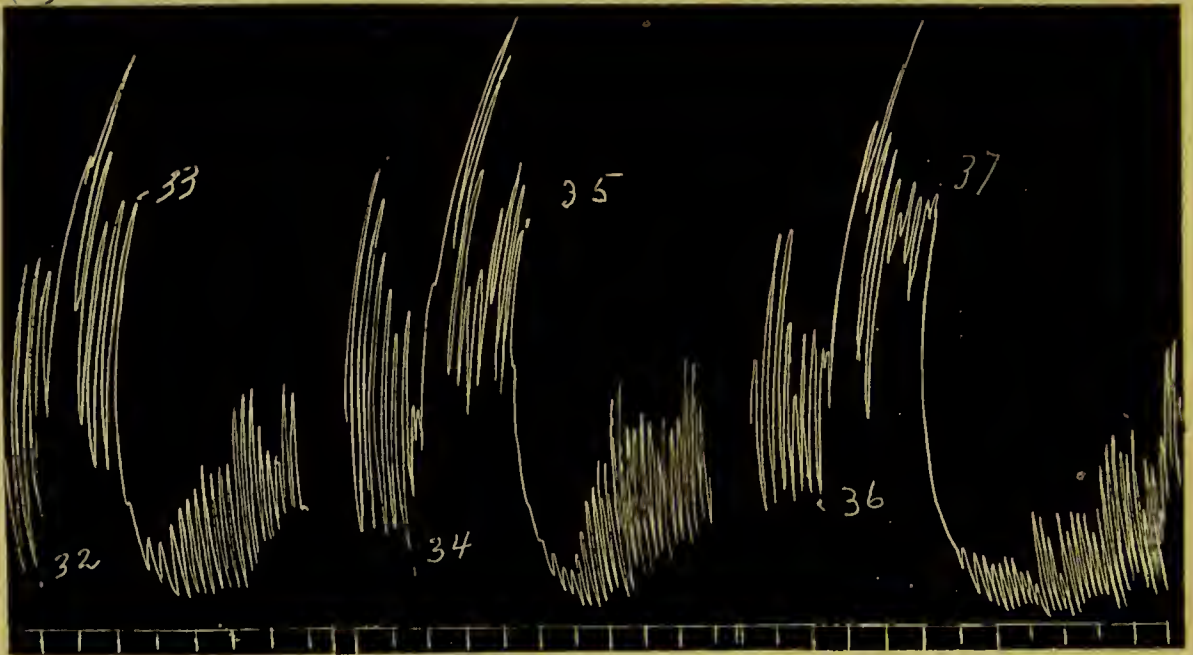


FIG. 13. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURE 12

At 32 Ringer was replaced by jugular blood and this at 33 by the third adrenal specimen (without asphyxia). At 34 Ringer was replaced by jugular blood and this at 35 by the fourth adrenal specimen (asphyxia). At 36 Ringer was replaced by jugular blood and this at 37 by the fifth adrenal specimen (without asphyxia). Bloods diluted with sixteen volumes Ringer. (Reduced to two-thirds.)

had a smaller concentration than 1:800,000, and a greater concentration than 1:1,100,000. This is as great a concentration as is ever found in the blood of the cat, collected and tested in this way.

The uterus tests (samples of the tracings are reproduced in figure 14) confirmed the results of the assay on the intestine segments. Observations 48, 50 and 51 show that the tone-increasing power of the fifth adrenal specimen was intermediate

between that of indifferent blood containing 1:1,600,000 adrenalin and that of indifferent blood containing 1:800,000 adrenalin. The third specimen (without asphyxia) produced only a slightly smaller increase of tone than the fourth specimen (collected during asphyxia). The second specimen produced a much smaller effect than any of the succeeding samples, corresponding to its greater rate of flow.

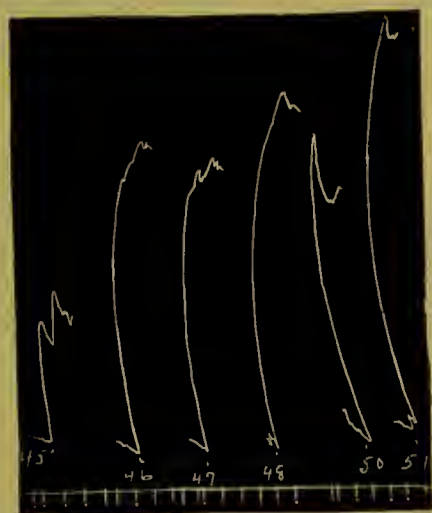


FIG. 14. UTERUS TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURES 12 AND 13

Ringer was replaced at 45 by the second adrenal specimen (without asphyxia); at 46 by the fourth specimen (asphyxia); at 47 by the third specimen (without asphyxia); at 48 by the fifth specimen (without asphyxia). Bloods diluted with sixteen volumes Ringer. Ringer was replaced at 50 by adrenalin in jugular blood (1:1,600,000); at 51 by adrenalin in jugular blood (1:800,000). The adrenalin was added to the undiluted bloods to the concentrations mentioned, the mixtures being then diluted with 16 volumes Ringer before application to the segment. (Reduced to one-half.)

The output of epinephrin per minute, assuming that the fifth sample had a concentration of 1:1,000,000, would be 0.0004 mgm. (0.0002 mgm. per kilo of animal per minute), which is about the same as is found in cats under the experimental conditions, but anesthetized with urethane without increased intracranial pressure (6). In this experiment, accordingly, neither the absence of a chemical anesthetic nor the increased intracranial pressure seems to have diminished the output.

This conclusion is supported by experiment 7, a control experiment in which urethane was combined with increased intracranial pressure.

Experiment 7. Condensed Protocol. Cat. Weight 2.425 kgm.

9.20 a.m. 5 grams urethane.

10.00 a.m. Tracheal and jugular cannulae inserted and jugular blood obtained. Trephined and inserted pressure bag. "Short" cava pocket made, renal, coeliac and mesenteric arteries and abdominal aorta being tied. The following samples of adrenal blood were now obtained, with no pressure in the intracranial bag.

1st sample: 0.9 gram in 20 seconds (2.7 grams per minute).

2nd sample: 1.9 grams in 1 minute, 30 seconds (1.3 grams per minute).

Pressure in the bag was now got up, to 250 mm. of mercury, and the following samples of adrenal blood collected:

3d sample: 2.6 grams in 2 minutes, 10 seconds (1.3 grams per minute).

4th sample: 4.3 grams in 3 minutes, 20 seconds (1.3 grams per minute).

5th sample: 3.3 grams in 3 minutes (1.1 grams per minute).

6th sample: 4.0 grams in 4 minutes (1.0 gram per minute).

Combined weight of adrenals 0.390 gram.

The adrenalin assay showed that the second adrenal blood specimen contained approximately 1:2,000,000 epinephrin, corresponding to an output per minute for the animal of 0.00065 mgm., or 0.00025 mgm. per kilogram of body weight per minute, an output within the range observed in cats anaesthetised with urethane, without increased intracranial pressure.³ A comparison of the epinephrin concentration in specimens of the adrenal blood collected without and with increased intracranial pressure revealed no difference in the rate of output. Thus,

³ See table 2, Journ. Pharm. and Exp. Therap., 1917, x, 4.

the second specimen caused an inhibition of the intestine segment only slightly less than that caused by the sixth specimen, corresponding to the somewhat greater blood flow when the second was being collected.

Since the tests instituted on adrenal blood directly collected from the cava pocket had failed to yield unequivocal evidence of an increase in the rate of liberation of epinephrin associated with asphyxia, it did not seem probable that observations on the highly diluted adrenal blood obtained from the inferior cava central to the orifices of the adrenal veins (7) would reveal a difference. Nevertheless, the catheter method was tried, but again with a negative result. In one experiment, the condensed protocol of which is published elsewhere (8), a comparison of cava blood drawn off by a catheter from above the level of the adrenals during asphyxia was made with blood similarly obtained during stimulation of the sciatic nerve, and with blood collected through the catheter in the absence both of asphyxia and sensory stimulation. All these bloods behaved in the same way towards rabbit intestine and uterus segments. There was also no distinct difference between the action of any of them, and that of indifferent blood collected from the lower part of the inferior cava. It was proved that the reason for this was the dilution of the adrenal contribution by indifferent cava blood. The degree of dilution with indifferent blood necessary to render undetectable pure adrenal blood collected from the cava pocket, and containing a content of epinephrin within the ordinary range was ascertained. It was shown to fall within the limits of the dilution which the adrenal blood must normally undergo in the cava. We do not see how it is possible to make quantitative comparisons of the rate of output of epinephrin under different conditions by the catheter method.

CONCLUSION

An attempt was made to determine whether asphyxia produces a detectable increase in the rate of liberation of epinephrin from the adrenals, as determined by testing adrenal vein blood on rabbit intestine and uterus segments. The result was negative.

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EFFECT OF STIMULATION OF SENSORY NERVES UPON THE RATE OF LIBERATION OF EPINEPHRIN FROM THE ADRENALS.

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PLATES 48 TO 53.

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The fact that the spontaneous liberation of epinephrin is dependent upon the integrity of certain efferent nerves running in the sympathetic system has led to attempts to influence the rate of liberation reflexly. We have previously¹ published experiments in which the rates of liberation of epinephrin during and without stimulation of brachial nerves (in the cat) were compared by means of the denervated eye reactions of Meltzer and by the rise of blood pressure produced when adrenal blood collected for a known time in a cava pocket is released. The results were negative.

We have since repeated the experiments on cats and dogs, drawing off blood from the cava pocket and testing it on rabbit intestine and uterus segments. In this way the adrenal blood can, of course, be applied to the test-objects without dilution if so desired, whereas, with the eye and blood pressure reactions, it is necessarily much diluted. On the other hand, the use of the latter reactions has some great advantages. The blood is not withdrawn from the vessels, and accordingly there is no danger of loss of a part of the epinephrin in the necessary manipulations before the blood is applied to the segments. The development of the pressor property of clotted blood, a serious complication for some of the methods of testing extravascular blood (frog perfusion, artery rings), is avoided. Possible effects upon the rate of liberation of the loss of blood, or even of the loss of the epinephrin in it when withdrawn from the body are also excluded. Most impor-

¹ Stewart, G. N., and Rogoff, J. M., *J. Pharm. and Exp. Therap.*, 1916, viii, 517.

tant of all, the rise of blood pressure, especially if interpreted by the aid of the eye reactions, affords a direct quantitative comparison of the amount of epinephrin liberated in successive observations.

Technique.—The adrenal blood was collected through a boiled and oiled cannula in the inferior cava. The cava pocket was usually much shorter than that employed for the eye and blood pressure reactions, where it was essential to have as roomy a pocket as possible. With the short pocket only a small dead space is left filled with blood which has passed through the adrenals during one set of experimental conditions at the moment when the experimental conditions have been changed. To reduce still further any overlapping of the blood samples successively collected with and without stimulation, excitation of the sensory nerve was started slightly before completion of the collection of the preceding "no stimulation" sample, and *vice versa*. Once begun, the flow of blood from the cannula was interrupted, sample after sample being collected. The time of collection and the weight or volume of blood being accurately measured, the rate of blood flow during the collection of each sample is known. This is indispensable, of course, for estimating the rate of liberation of epinephrin by reactions which, like the segment tests, give only the concentration. Since, as we have found,² the concentration in successive samples tends to increase as the blood flow slackens, we varied the order of stimulation and no stimulation observations, interposing for example, a stimulation period between two no stimulations, and *vice versa*. Richards and Wood,³ in their work on the influence of stimulation of the depressor upon suprarenal secretion, have recognized the necessity of measuring the rate of flow of the blood, and the advisability of not taking the samples in a uniform order. The stock adrenalin used for the epinephrin assays was always freshly assayed by the colorimetric method of Folin, Cannon, and Denis.⁴

In our previous observations we stimulated the brachial nerves, as it was convenient in forming the cava pocket to clamp the abdominal aorta, and the sciatic nerve was therefore not available. Since the upper thoracic portion of the spinal cord can sustain a substantial liberation of epinephrin after section of the cord in the cervical region,⁵ the sciatic might perhaps be considered more likely to yield positive effects than the brachial. Elliott's conclusion⁶ that after section of the brain stem just in front of the anterior corpora quadrigemina exhaustion of the epinephrin store of the adrenal by stimulation of sensory nerves occurs, while it does not take place if the cord has been cut just below the bulbar vasomotor center, does not, as already pointed out,¹ necessarily indicate that the center concerned in epinephrin liberation, on which Elliott made no experiments, is as

² Stewart and Rogoff, *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 77.

³ Richards, A. N., and Wood, W. G., *Am. J. Physiol.*, 1915-16, xxxix, 54.

⁴ Folin, O., Cannon, W. B., and Denis, W., *J. Biol. Chem.*, 1912-13, xiii, 477.

⁵ Stewart and Rogoff, *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 143.

⁶ Elliott, T. R., *J. Physiol.*, 1912, xlv, 374.

high as this. But if it were, brachial stimulation ought to hit it, as well as sciatic stimulation. To be sure, however, of stimulating afferent nerves favorably situated with reference to one or the other of the centers concerned, if there is more than one, we used both sciatic and brachial in these experiments.

While cats proved suitable for the previous observations in which blood was not withdrawn, it was judged advisable in the present series to use dogs also, in order to make certain of a good and uninterrupted flow of blood, which would completely wash out the pocket and cannula, and thus prevent overlapping of the successive samples.

Experiment 1. Condensed Protocol.—Dog; weight 10 kilos. Ether anesthesia. 45 cc. of blood withdrawn from jugular vein. Cava pocket tied off. Intestinal arteries and abdominal aorta not tied. Right iliac artery ligated and cannula inserted into the right iliac vein. The left iliac vein was not tied, and was clamped just before the collection of blood was begun, in order to spare the left sciatic as much as possible. The left sciatic and brachial nerves were prepared for stimulation. The following blood specimens were collected from the cannula in the iliac.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		cc.	
1	13.0	1 min., 40 sec.	8.0	None.
2	17.3	2 " 25 "	7.2	Brachial.
3	9.2	1 " 40 "	5.8	"
4	7.4	1 " 47 "	4.4	None.
5	10.2	3 " 10 "	3.2	Sciatic.
6	7.5	3 " 10 "	2.4	"
7	5.0	3 " 24 "	1.5	None.

While the pocket was still clipped off, 53 cc. of arterial blood were obtained from the carotid artery. Combined weight of adrenals 1.050 gm.

It will be seen from Figs. 1 to 3, that no difference could be made out between the inhibitory effects produced on the rabbit intestine segments by the various adrenal blood samples, which could be connected with the presence or absence of nerve stimulation. Thus in Fig. 1, the second sample collected during brachial stimulation (Observation 5) caused practically the same effect as the first sample, collected without stimulation, and the blood flows were about the same during collection of the two samples. The third sample, with nerve stimulation, gave a greater inhibition (Observation 7) than the first, without stimulation; but stimulation of the nerves had nothing

to do with this, since the nerves were also stimulated during collection of the second sample (Observation 5). The explanation of the greater inhibition produced by the third sample is that the blood flow was slower during its collection, and the rate of liberation of epinephrin per minute remaining the same, the concentration was necessarily greater. The adrenalin assays showed that the concentration in the first and second samples was somewhat more than 1: 3,300,000, corresponding to an output of epinephrin per minute of 0.0025 mg. (0.00025 mg. per kilo of body weight per minute), and that the third specimen was not twice as strong as the first or second. This is a normal output for a dog under the experimental conditions, as estimated in drawn adrenal blood on rabbit intestine segments.

In Fig. 2 it is shown that sciatic stimulation also produced no demonstrable effect, the slight preponderance of the fifth sample (Observation 15) as compared with the fourth (Observation 13) being plainly associated with the somewhat slower flow during collection of the latter. In Fig. 3 the sixth and seventh adrenal samples were compared with greater magnification; but the result was the same; the blood collected during stimulation of the sciatic caused no greater effect than that collected without stimulation. It is probable, however, that the concentration of epinephrin in the sixth and seventh samples was such as to produce the maximum inhibition of which the segment was capable at the time. That the sixth specimen really contained somewhat less epinephrin than the seventh, corresponding to the greater blood flow, is brought out by the uterus tests (Fig. 4, Observations 48 and 50). The same progressive increase in concentration in the successive samples shown by the intestine tracings is exhibited on the uterus tracings, only, as is commonly the case, even more sharply. The difference between the fourth and third specimens (Observations 44 and 45) would unquestionably have been brought out clearly with a greater degree of dilution, as the increase of uterus tone in these observations was already approaching, if it had not indeed reached the maximum for the segment.

Experiment 2. Condensed Protocol.—Cat; weight 2.9 kilos. Urethane anesthesia. Obtained specimen of jugular blood, then isolated left sciatic nerve and prepared it for stimulation. Cava pocket made, tying off intestinal and renal vessels and right iliac artery. Cannula inserted into the cava, making a short pocket. Adrenal blood was then collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	gm.		gm.	
1	1.5	1 min., 25 sec.	1.1	None.
2	4.4	4 "	1.1	Sciatic.
3	1.4	6 "	0.23	None.
4	1.8	9 " 30 "	0.19	"
5	2.3	13 "	0.18	Sciatic.
6	1.7	8 "	0.21	None.
7	2.2	10 "	0.22	"

During stimulation of the sciatic, the pupils dilated widely, the respiratory movements were increased, and there were reflex movements indicating that the stimulus was effective. Stimulation was begun $\frac{1}{2}$ to 1 minute before collection of the corresponding specimen, and was stopped $\frac{1}{2}$ to 1 minute before the collection of the specimen ceased, to allow for washing out of the dead space. The flow was notably diminished after the first period of strong stimulation; that is, after the collection of the second specimen. After the last adrenal sample was collected indifferent blood was obtained from the abdominal aorta. Combined weight of the adrenals 0.347 gm.

Specimens of the tracings with rabbit segments are given in Figs. 5 to 7. They show the same general result as in Experiment 1; that is, a progressive increase in epinephrin concentration in successive adrenal samples, unmodified within the limits of sensitiveness of the method by stimulation of the sciatic.

Experiment 3. Condensed Protocol.—Cat; weight 2.58 kilos. Urethane anesthesia. Cava pocket prepared with cannula in left renal vein. Renal and intestinal arteries tied, but not the abdominal aorta. Sciatic nerve prepared for stimulation. Adrenal blood samples were collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		cc.	
1	10.0	5 min.	2.0	None.
2	9.0	6 " 30 sec.	1.5-2.0*	Sciatic.
3	10.3	5 "	2.0	None.
4	10.0	—	—†	During asphyxia.

* Part of the specimen was lost by accidentally spilling from the container.

† The blood clotted in the cannula at the end of the collection, and was gradually slowing so that the flow per minute could not be properly calculated.

Indifferent blood was obtained from the abdominal aorta. Indifferent blood from the carotid of another cat was also used in the tests after testing it against the blood from the abdominal aorta, and finding it to have the same tone-increasing power. Combined weight of the adrenals 0.328 gm.

The same results were obtained with the intestine and uterus segments as in the preceding experiments; there was equality or a progressive increase of epinephrin in the successive adrenal blood samples according to whether the blood flow remained constant or slackened. The output of epinephrin per minute was not modified, within the limits of accuracy of the assays, by stimulation of the sciatic. The concentration of epinephrin in the second and third adrenal specimens was about the same (1:3,500,000) corresponding to a liberation of 0.0006 mg. per minute (more than 0.0002 mg. per kilo of body weight per minute), a normal output as estimated on drawn adrenal blood by rabbit segments. The uterus segments could detect a concentration of 1:24,000,000 adrenalin in indifferent blood as compared with the indifferent blood itself.

Experiment 4. Condensed Protocol.—Dog; weight 8.5 kilos. Urethane and ether anesthesia. Cava pocket prepared, tying off the renal and intestinal arteries. The abdominal aorta was not clamped till just before the collection of blood. Cannula in right iliac vein. Left sciatic prepared for stimulation. About 5 cc. of blood were first collected from the pocket and discarded so as to wash the pocket free of any epinephrin which might have been liberated in the manipulations. The following adrenal samples were then collected.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		cc.	
1	34.0	2 min., 30 sec.	13.6	Sciatic.
2	24.0	3 "	8.0	None.
3	22.0	3 "	7.3	"
4	25.0	4 "	6.2	Sciatic.

Serum was obtained from the bloods by centrifugalization. The corpuscle sediment was about one-third of the total volume.

In this experiment serum was used for the segment tests instead of blood, so as to increase the chance of detecting any difference due to stimulation, since serum contains a greater concentration of epi-

nephrin than the blood from which it is derived.⁷ The result was the same as in the other experiments. Thus, the second adrenal specimen, collected without stimulation (Observation 15, Fig. 8) had a somewhat greater inhibitory effect upon the intestine segments than the first specimen collected during sciatic stimulation (Observation 11), corresponding to the difference in blood flow. The effect of the third specimen (Observation 6, Fig. 9) is greater than that of the second (Observation 4), and less than that of the fourth specimen (Observation 9), without apparent relation to the presence or absence of nerve stimulation. The adrenalin assay showed that the second specimen contained about 1:3,500,000 epinephrin, corresponding to 1:5,000,000 for the blood, an output per minute of 0.0016 mg. (0.0002 mg. per kilo of body weight per minute), a normal output for a dog, as estimated in this way. As this is a moderate concentration, and the first specimen contained still less, the failure of nerve stimulation to increase the concentration could not have been due to the initial concentration being near the possible maximum. The serum of the third specimen contained 1:3,000,000 epinephrin, corresponding to 1:4,300,000 for the blood. This gives the same output as for the second specimen (0.0017 mg. per minute), the concentrations being inversely proportional to the blood flows. The uterus tracings, some of which are reproduced in Fig. 10, confirm the conclusion that the first adrenal specimen (Observations 31 and 33) contained a smaller concentration of epinephrin than the second (Observations 32 and 34), although the sciatic had been stimulated during the collection of the first. Observation 30, Fig. 10, shows a much smaller effect with indifferent serum, compared with any of the adrenal sera, thus confirming the conclusion that the substance inhibiting the intestine was epinephrin.

The objection might be made that under the influence of the experimental conditions (anesthesia, trauma, etc.), the rate of liberation of epinephrin might be already so great that it could not be augmented by stimulation of afferent nerves. This objection has already been partly met by the fact that with moderate concentrations of epinephrin, as shown by adrenalin assays, nerve stimulation fails to

⁷ Stewart and Rogoff, *J. Pharm. and Exp. Therap.*, 1916-17, ix, 393.

increase the concentration. There is no evidence that anesthetics increase the liberation demonstrably. In cats, some days after section of the spinal cord in the cervical region, we have found⁴ that blood collected from the adrenal veins through a cannula in the inferior cava contains concentrations of epinephrin within the ordinary range, despite the fact that on account of the complete anesthesia below the level of the cord section it was not necessary to give an anesthetic. Elliott's result, that anesthetics cause diminution of the epinephrin store of the adrenals, is no proof, even if we admit that the diminution is really due in some direct way to the anesthetic, that the output of epinephrin is increased under their influence; since a diminution in the rate of formation of epinephrin would equally be accompanied by a diminution in the store.

Nevertheless, we made a number of experiments in which the animal was rendered insensitive by destruction of the cerebral cortex, or by increase of intracranial pressure without the use of anesthetics, except for a few minutes while the brain was being destroyed or the skull trephined for the insertion of the pressure bag.

Experiment 5. Condensed Protocol.—Dog; weight 7.6 kilos. Animal rendered insensible by destruction of the cerebral cortex with much of the underlying centrum ovale. It was shown at autopsy that none of the brain tissue behind the anterior edge of the anterior corpora quadrigemina had been destroyed. Ether was given only during destruction of the brain. Indifferent blood was obtained from the jugular vein. Cava pocket made. Right iliac artery, and intestinal and renal arteries tied off. Left sciatic nerve prepared for stimulation. Adrenal blood specimens collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	<i>gm.</i>		<i>gm.</i>	
1	3.5	1 min.	3.5	None.
2	7.0	3 "	2.3	"
3	7.7	4 " 30 sec.	1.7	Sciatic.

A fourth adrenal specimen was obtained without stimulation, but clotting in the cannula prevented accurate measurement of the time (3 gm. in 4 to 5 minutes). Capacity of the cava pocket, which was a long pocket, 0.9 to 1.0 gm. of blood.

In this animal no evidence was forthcoming, any more than in the others, that stimulation of the sciatic was associated with a detectable increase in the rate of epinephrin output. A few of the tracings are reproduced in Fig. 11. The second adrenal specimen, collected without stimulation (Observation 23), caused about the same amount of inhibition of the intestine as the third specimen, collected during sciatic stimulation (Observation 25). If anything, the effect of the second was somewhat greater than that of the third specimen. The flows were not very different for the two specimens. The fourth adrenal specimen (Observation 19), collected without sciatic stimulation, produced a decidedly greater effect than the third, corresponding to the much slower flow. Here is an instance where a nerve stimulation period between two periods without stimulation ought to have shown some change of concentration, as compared either with the preceding or the succeeding period, had the nerve stimulation been capable of evoking such a change. The first adrenal specimen, without nerve stimulation (Observation 21), has a greater inhibitory effect than either the second or third. But this is doubtless due to epinephrin liberated during manipulations while the pocket was being tied off. Although it is often possible to complete the operation without any evidence of manipulative discharge, yet, in order to be sure of avoiding errors due to this cause, the first specimen was always considered suspect, if it gave a higher concentration than the second, and in that case rejected. It was, in fact, collected separately for the purpose of insuring that the succeeding specimens should not contain any epinephrin liberated by massage, etc., during the formation of the pocket.

Uterus tracings confirmed the conclusion that the second and third adrenal specimens (Experiment 5) had about the same concentration of epinephrin, and that the fourth had a greater concentration than either the second or third. Adrenalin assays showed that the concentration in the third specimen was about 1: 3,000,000, corresponding to an output of 0.0006 mg. of epinephrin per minute (about 0.0001 mg. per kilo of body weight per minute). This is rather below than above the average output in anesthetized dogs, as estimated by rabbit segments.

Elliott has stated that brain injuries such as destruction of the cerebral hemispheres cause discharge of the epinephrin store of the adrenals. Although for the reason already mentioned, this would not of itself be sufficient proof that the rate of liberation of epinephrin is sensibly increased by the brain irritation, an experiment was made in which the animal was rendered insensitive by increasing the intracranial pressure, without any brain mutilation, by a thin rubber bag introduced through a trephine hole.

Experiment 6. Condensed Protocol.—Dog; weight 9.4 kilos. Under ether anesthesia the skull was trephined, and a rubber bag inserted. The bag was connected with a mercury manometer. The pressure in it was increased to 250 mm. of mercury, and the ether discontinued. Artificial respiration was started, although the dog was still breathing well spontaneously. A short cava pocket was made. The abdominal aorta was tied off below the renals, and the renal vessels were tied. Brachial nerves on one side prepared for stimulation. As the blood pressure fell the intracranial pressure was diminished. Adrenal blood was collected as follows: The first specimen (5 to 6 gm.) was rejected to avoid any epinephrin liberated during manipulation.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	gm.		gm.	
2	7.2	45 sec.	9.0	None.
3	12.0	1 min., 35 "	8.0	Brachial.
4	12.5	2 " 20 "	5.4	None.
5	9.2	2 " 30 "	3.7	Brachial.

Combined weight of adrenals 1.20 gm.

In Fig. 12 are reproduced a few of the intestine segment tracings from Experiment 6. They show that the third adrenal specimen, collected during brachial stimulation (Observation 31), while causing a somewhat greater inhibition than the second specimen, collected without stimulation (Observation 29), does not produce as great an inhibitory effect as the fourth specimen, collected without stimulation. The fifth adrenal specimen, collected during brachial stimulation (Observation 35), causes an inhibition not conspicuously different from that caused by the fourth specimen. As before, the progressive increase in epinephrin concentration associated with the gradual de-

cline in the rate of blood flow has not been sensibly altered by the nerve stimulation. This result was confirmed by the uterus tracings, some of which are reproduced in Fig. 13. If the third adrenal specimen (Observation 52) were compared merely with the second (Observation 51), it might be thought that the greater effect on the uterus produced by the third specimen indicated an increased output of epinephrin due to stimulation of the nerve. This conclusion is at once seen to be erroneous when we compare the effect of the fourth specimen (Observation 54) with that of the third, for the fourth is as much stronger than the third as the third is stronger than the second, and the nerves were not being stimulated during collection of the fourth specimen. To show the uniformity of the tracings, Observation 52 on the third specimen was interposed between two observations (51 and 53) on the second. In Observations 56 to 59, the second to fifth specimens were compared in a greater dilution, and just as in the case of the intestine tracings no such preponderance of effect was revealed in the third and fifth specimens as would be expected if the nerve stimulation during their collection had sensibly increased the output of epinephrin.

Adrenalin assays were made on the intestine, and in this case also on the uterus segments. The intestine segments are more generally useful than the uterus for assaying the concentration of epinephrin, although for bringing out qualitative differences the uterus is frequently much more sensitive than the intestine. But with favorable uterus segments good quantitative results are also obtained. The uterus from adult or nearly adult virgin rabbits is the best in our experience for all such work. The assays indicated that the second adrenal specimen contained about 1:9,000,000 epinephrin (Fig. 13, Observations 61, 64, 65, and 66); and the third specimen more than 1:9,000,000 but much less than 1:6,000,000 (Fig. 13, Observation 68), probably not far from 1:8,000,000. The fourth specimen had a greater concentration than 1:8,000,000, though distinctly less than 1:5,000,000, and somewhat less than 1:6,000,000, probably about 1:6,500,000. If the blood flows in the protocol are compared with these concentrations, it will be seen that the concentrations are approximately in the inverse ratio of the flows. In other words, during the collection of these adrenal samples, the output of epinephrin per minute (0.001 mg. or more

than 0.0001 mg. per kilo of body weight per minute) remained approximately constant, and was not sensibly modified by stimulation of the brachial nerve.

It did not seem probable that if negative results were yielded by unmixing adrenal vein blood, positive results would be obtainable with cava blood collected by a catheter anterior to the adrenal veins.⁸ However, as the necessary operation for the catheter method is less severe than for the cava pocket method, the possibility could not be overlooked that the output of epinephrin in the periods without nerve stimulation might be less when extensive trauma was avoided. If this were so, an increase in the liberation produced by the stimulation of nerves might more readily make itself felt. Of course, it is impossible by this method to take account of any changes in the rate of blood flow through the adrenals; and comparisons of the concentration of epinephrin in the blood are only valid for the estimation of changes in the rate of liberation if alterations in the rate of blood flow are known, unless the assumption can be made that the blood flow remains constant during the whole experimental period. Nevertheless, the conditions which in the abdominal operation lead to notable alterations in the rate of blood flow were not so likely to be present with the catheter method, except in so far as the catheter itself might interfere with the flow of blood in the cava, and therefore we made a number of experiments in this way. Experiment 7 is an example.

Experiment 7. Condensed Protocol.—Cat; weight 2.875 kilos.

10.00 a.m. 5 gm. urethane by stomach tube.

11.00 a.m. Exposed left femoral vein and prepared it for catheter; prepared right sciatic nerve for stimulation.

11.20 a.m. Blood I obtained through catheter inserted to a level just anterior to adrenal veins. At autopsy, the eye of the catheter was found to be about 5 to 6 mm. anterior to the orifice of the right adrenal vein when inserted to the distance used in the experiment.

11.32 a.m. Started stimulation of sciatic.

11.37 a.m. Blood II collected through catheter at the same level, sciatic stimulation being continued throughout the collection.

11.40 a.m. Blood III obtained from lower cava through catheter, which was withdrawn 9 cm.

⁸ Cannon, W. B., and Hoskins, R. G., *Am. J. Physiol.*, 1911-12, 'xxix, 274.

12.00 m. Blood IV obtained from catheter in the same manner as Blood I.

12.05 p.m. Blood V obtained from catheter anterior to the adrenal veins, collected during asphyxia.

12.09 p.m. Blood VI obtained from catheter lower down in cava, as for blood III.

After obtaining each of the bloods I, III, and IV, the catheter was withdrawn, cleaned, and oiled again. The bloods were withdrawn as uniformly as possible by the aid of an aspirator.

The result was negative. No difference was found by rabbit intestine and uterus segment tests between blood withdrawn without stimulation of the sciatic, and blood withdrawn during stimulation. None of the samples caused any inhibition of the intestine segments. We do not doubt that under favorable conditions (especially sensitive segments, slow blood flow in the inferior cava, and possibly a fortunate location of the eye of the catheter with reference to the adrenal vein orifices) samples of blood may sometimes be drawn from the inferior cava containing a sufficient concentration of epinephrin to yield distinct reactions. We failed to obtain such reactions because the epinephrin given off by the adrenals in the usual amount was too highly diluted by the cava blood. To illustrate the effect of this dilution we made some experiments, in which pure adrenal blood from the cava pocket and catheter blood from above the level of the adrenals, obtained from the same animal, were compared. In some observations catheter blood from the level of the adrenals collected while the adrenal veins were clipped was compared with blood from the same level collected through the catheter with the adrenal veins open, also with a negative result. Experiment 8 is an example of these experiments.

Experiment 8. Condensed Protocol.—Cat; weight 2.68 kilos. Urethane 3 gm. by stomach tube, and later on, 2 gm. more. Both adrenal veins isolated and prepared for clipping. Femoral vein prepared for catheter insertion. Blood I, drawn through catheter from level just anterior to adrenal veins; blood II, from catheter at the same level, but with adrenal veins clipped; blood III, obtained in same manner as blood I; blood IV (10 cc. in 11 minutes) obtained from cava pocket through cannula in right renal vein. After releasing the pocket by removing the clamps, blood V was obtained through the catheter in the same way as blood I. Indifferent (arterial) blood was obtained from the abdominal aorta. The catheter was withdrawn, cleaned, and oiled after each specimen was collected.

In Fig. 14 it is seen that catheter blood from the level of the adrenals displacing arterial blood (Observation 10) produced no inhibition of the intestine segment, although pure adrenal vein blood (Observation 8) caused good inhibition. Adrenalin assays showed that the concentration of epinephrin in the adrenal blood was not far from 1:3,000,000. It was confirmed on uterus segments (Fig. 15, Observations 33, 35, and 45) that the catheter blood caused no greater effect than indifferent blood, and much less than adrenal blood (Observations 34 and 36). The uterus segment, as it happened, gave practically no increase of tone with indifferent blood, which rendered the demonstration of the absence of detectable epinephrin in the catheter blood all the more convincing. Catheter blood collected with clipped adrenal veins (Observation 37) behaved in no respect differently from blood similarly collected, but with the adrenal veins open (Observation 39). The segment could easily detect epinephrin in the concentration of 1:65,000,000 (Observation 46). From the relatively considerable increase of tone given by blood with this concentration of adrenalin, there is no doubt that a much smaller concentration could have been detected. Accordingly, the adrenal vein blood must have been diluted in the inferior cava much more than twenty times.

In Fig. 16 (Observation 20) catheter blood from the level of the adrenals, collected with the adrenal veins clipped, was displaced by catheter blood collected with the veins open. No change in the intestine segment curve was produced; that is, the adrenal blood was so much diluted in the cava that a sample of cava blood containing the adrenal contribution could not be discriminated by this intestinal segment from a sample of cava blood which could not have been mixed with any adrenal blood. Nor could it be discriminated from the indifferent (arterial) blood, since its replacement by this (Observation 21) left the curve unaffected. A prompt and marked inhibition was produced, however, when the arterial blood was in its turn displaced by the pure adrenal blood (Observation 22).

In the last experiment to be quoted (Experiment 9), blood was collected by a catheter at the level of the adrenals during stimulation of the sciatic and without sciatic stimulation. Pure adrenal blood was also collected from the same dog, during and without stimulation of the sciatic.

Experiment 9. Condensed Protocol.—Dog; weight 14.9 kilos. Ether anesthesia. A specimen of indifferent blood was collected from the jugular vein. Then the left femoral vein was prepared for insertion of the catheter, and the right sciatic nerve prepared for stimulation. The catheter was now inserted to a level just anterior to the adrenal veins. At autopsy it was verified, as in all the other experiments of this type, that the eye of the catheter was anterior to the orifices of the adrenal veins. Three samples of blood were now collected through the catheter, the first without sciatic stimulation (13.0 cc. in 1 minute and 30 seconds), the second during sciatic stimulation (13.8 cc. in 1 minute and 56 seconds), and the third during sciatic stimulation (12.6 cc. in 1 minute and 18 seconds). The catheter was now removed, washed, oiled again, and reinserted to the same level after an interval of 6 minutes; and a fourth specimen collected through the catheter without sciatic stimulation (23.5 cc. in 3 minutes and 12 seconds). A cava pocket was now made, the renal and left iliac vessels being tied, and a cannula inserted in the left iliac vein. The abdominal aorta was clamped at the bifurcation just before beginning the collection of blood from the pocket. Adrenal blood samples were obtained from the pocket as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.	Stimulation.
	cc.		cc.	
1	15.2	1 min., 50 sec.	8.4	None.
2	16.1	2 " 5 "	8.0	Sciatic.
3	18.3	2 " 32 "	7.0	"
4	20.0	2 " 30 "	8.0	None.
5	22.9	3 " 55 "	5.7	"
6	11.4	3 " 45 "	3.0	Sciatic.
7	13.1	4 " 5 "	3.2	"
8	7.1	4 "	1.8	None.

Combined weight of adrenals, 1.15 gm.

The bloods were carefully compared on intestine and uterus segments and the degree of dilution of the adrenal blood with indifferent blood, which just caused the inhibitory effect on the intestine segments to become too slight to be detected with certainty, was determined. In Fig. 17 some of the tracings are given. At 8 the fifth adrenal blood replaced indifferent blood, causing a marked inhibition of the intestine segment. The concentration of epinephrin in this adrenal sample was assayed at about 1:3,000,000, corresponding to an output of 0.002 mg. of epinephrin per minute (0.00013 mg. per kilo of body weight per minute). In the eighth specimen the concen-

tration was assayed at 1:1,100,000, about three times as great as in the fifth sample. This is approximately the inverse ratio of the blood flows, the rate of output of epinephrin being practically unchanged. At 24 blood collected with a catheter at the level of the adrenals during sciatic stimulation replaced indifferent blood. It produced no inhibition, but instead a further increase of tone. Observations 26 and 28 represent the effect of the fifth adrenal blood specimen diluted respectively with ten and with twenty volumes of indifferent blood. Inhibition of the segment can still be detected in each case. Diluted with forty volumes of indifferent blood (Observation 30) the fifth adrenal specimen produced an inhibition so slight, if any, that it could not have been detected unless perhaps by comparison with the preceding and succeeding observations. With this dilution the concentration of epinephrin in the blood was already only 1:120,000,000, and this blood was again diluted with four volumes of Ringer's solution before being applied to the segment. At 32, the adrenal blood, diluted with eighty volumes of indifferent blood, replaced the indifferent blood. No inhibition could be clearly detected any more than with the catheter blood (Observation 24).

We fail to see how it is possible to make exact experiments on the rate of liberation of epinephrin by the catheter method.

SUMMARY.

An attempt was made to determine whether stimulation of afferent nerves (sciatic and brachial) produced a detectable increase in the rate of liberation of epinephrin from the adrenals, as determined by testing adrenal vein blood on rabbit intestine and uterus segments. The result was negative.

EXPLANATION OF PLATES.

In all the tracings time is marked in half minutes.

PLATE 48.

FIG. 1. Intestine tracings. Blood of a dog anesthetized with ether. At 2 Ringer's solution was replaced by jugular blood, and this at 3 by the first adrenal blood specimen, collected without stimulation of nerves. At 4 Ringer's solution was replaced by jugular blood, and this at 5 by the second adrenal blood specimen,

collected during stimulation of the brachial. At 6 Ringer's solution was replaced by jugular blood, and this at 7 by the third adrenal blood specimen, collected during brachial stimulation. Reduced one-third.

FIG. 2. Intestine tracings. Blood of the same dog as in Fig. 1. At 12 Ringer's solution was replaced by jugular blood, and this at 13 by the fourth adrenal specimen, collected without stimulation of nerves. At 14 Ringer's solution was replaced by jugular blood, and this at 15 by the fifth adrenal blood specimen, collected during sciatic stimulation. At 19 Ringer's solution was replaced by jugular blood, and this at 20 by the second adrenal specimen, collected during brachial stimulation. At 21 Ringer's solution was replaced by jugular blood, and this at 22 by the first adrenal specimen, collected without stimulation of nerves. Reduced one-third.

FIG. 3. Intestine tracings. Blood of the same dog as in Figs. 1 and 2. Greater magnification. At 24 Ringer's solution was replaced by jugular blood and this at 25 by the sixth adrenal specimen, collected during sciatic stimulation. At 27 Ringer's solution was replaced by jugular blood, and this at 28 by the seventh adrenal specimen, collected without stimulation of nerves. Reduced one-third.

PLATE 49.

FIG. 4. Uterus tracings. Blood of the same dog as in Figs. 1 to 3. At 41 Ringer's solution was replaced by arterial blood; at 42, by the first adrenal specimen, collected without nerve stimulation; at 43, by the second adrenal specimen, collected during brachial stimulation; at 44, by the third adrenal specimen, collected during brachial stimulation; at 45, by the fourth adrenal specimen, collected without nerve stimulation. In Observations 41 to 45 the bloods were diluted with four volumes of Ringer's solution. At 47 Ringer's solution was replaced by the fifth adrenal specimen, collected during sciatic stimulation; at 48, by the sixth adrenal specimen, collected during sciatic stimulation; at 50, by the seventh adrenal specimen, collected without nerve stimulation. In Observations 47 to 50 the bloods were diluted with nine volumes of Ringer's solution. Reduced one-half.

FIG. 5. Intestine tracings. Blood from a cat anesthetized with urethane. At 4 Ringer's solution was replaced by jugular blood, and this at 5 by the second adrenal blood specimen, collected during sciatic stimulation. At 6 Ringer's solution was replaced by jugular blood, and this at 7 by the fourth adrenal specimen, collected without sciatic stimulation. The bloods were diluted with four volumes of Ringer's solution. Reduced one-third.

FIG. 6. Intestine tracings. Blood from the same cat used for Fig. 5. At 12 Ringer's solution was replaced by jugular blood, and this at 13 by the seventh adrenal specimen, collected without stimulation of the sciatic. At 14 Ringer's solution was replaced by jugular blood, and this at 15 by the fifth adrenal specimen, collected during sciatic stimulation. The bloods were diluted with two volumes of Ringer's solution. Reduced one-third.

FIG. 7. Uterus tracings. Blood of the same cat used for Figs. 5 and 6. At 28 Ringer's solution was replaced by jugular blood; at 29, by the second adrenal blood specimen, collected during sciatic stimulation; at 30, by the fourth adrenal specimen, collected without stimulation; at 31, by the fifth adrenal specimen, collected during sciatic stimulation; at 32, by the seventh adrenal specimen, collected without stimulation. All the bloods were diluted with four volumes of Ringer's solution. Reduced one-half.

PLATE 50.

FIG. 8. Intestine tracings. Sera of a dog anesthetized with urethane and ether. At 10 Ringer's solution was replaced by serum of arterial blood, and this at 11 by serum of the first adrenal specimen, collected during sciatic stimulation. At 14 Ringer's solution was replaced by serum of arterial blood, and this at 15 by serum of the second adrenal specimen, collected without sciatic stimulation. Reduced one-third.

FIG. 9. Intestine tracings. Sera of the same dog as in Fig. 8. At 3 Ringer's solution was replaced by serum of arterial blood, and this at 4 by serum of the second adrenal specimen, collected without nerve stimulation. At 5 Ringer's solution was replaced by serum of arterial blood, and this at 6 by serum of the third adrenal specimen, collected without nerve stimulation. At 8 Ringer's solution was replaced by serum of arterial blood, and this at 9 by serum of the fourth adrenal specimen, collected during sciatic stimulation. Reduced one-third.

FIG. 10. Uterus tracing. Sera of the same dog used for Figs. 8 and 9. At 30 Ringer's solution was replaced by serum of arterial blood; at 31, by serum of the first adrenal specimen, collected during sciatic stimulation; at 32, by serum of the second adrenal specimen, collected without sciatic stimulation. The serum in Observation 30 was undiluted, in Observations 31 and 32 it was diluted with an equal volume of Ringer's solution. At 33 Ringer's solution was replaced by serum of the first adrenal specimen diluted with three volumes of Ringer's solution, and at 34 by serum of the second adrenal specimen similarly diluted. Reduced one-half.

PLATE 51.

FIG. 11. Intestine tracings. Blood of a dog rendered insensitive by destruction of the cerebral cortex. At 18 Ringer's solution was replaced by jugular blood, and this at 19 by the fourth adrenal specimen, collected without nerve stimulation. At 20 Ringer's solution was replaced by jugular blood, and this at 21 by the first adrenal specimen, collected without nerve stimulation. At 22 Ringer's solution was replaced by jugular blood, and this at 23 by the second adrenal specimen, collected without nerve stimulation. At 24 Ringer's solution was replaced by jugular blood, and this at 25 by the third adrenal specimen, collected during sciatic stimulation. All the bloods were diluted with four volumes of Ringer's solution. Reduced one-half.

FIG. 12. Intestine tracings. Bloods from a dog rendered insensitive by increased intracranial pressure. At 28 Ringer's solution was replaced by indifferent (arterial) blood, and this at 29 by the second adrenal specimen, collected without brachial stimulation. At 30 Ringer's solution was replaced by arterial blood, and this at 31 by the third adrenal specimen, collected with brachial stimulation. At 32 Ringer's solution was replaced by indifferent blood, and this at 33 by the fourth adrenal specimen, without brachial stimulation. At 34 Ringer's solution was replaced by arterial blood, and this at 35 by the fifth adrenal specimen, collected with brachial stimulation. All the bloods were diluted with two volumes of Ringer's solution. Reduced one-third.

PLATE 52.

FIG. 13. Uterus tracings. Bloods from the same dog used in Fig. 12. At 50 Ringer's solution was replaced by indifferent (arterial) blood; at 51, by the second adrenal specimen, collected without brachial stimulation; at 52, by the third adrenal specimen, collected with brachial stimulation; at 53, by the second adrenal specimen; at 54, by the fourth adrenal specimen, collected without brachial stimulation. All these bloods were diluted with two volumes of Ringer's solution. At 56 Ringer's solution was replaced by the fourth adrenal specimen; at 57, by the third adrenal specimen; at 58, by the second adrenal specimen; at 59, by the fifth adrenal specimen, collected with brachial stimulation. These bloods were diluted with four volumes of Ringer's solution. At 61 Ringer's solution was replaced by indifferent (arterial) blood made up with adrenalin to a concentration of 1:8,000,000; at 66, by indifferent blood made up with adrenalin to a concentration of 1:9,000,000; at 68, by the indifferent blood with adrenalin to a concentration of 1:6,000,000. All the adrenalin bloods were diluted with four volumes of Ringer's solution before application to the segment. At 64 Ringer's solution was replaced by the second adrenal specimen, at 65 by the third adrenal specimen, each diluted with four volumes of Ringer's solution. Reduced one-half.

FIG. 14. Intestine tracings. Blood from a cat anesthetized with urethane. At 7 Ringer's solution was replaced by indifferent (arterial) blood, and this at 8 by adrenal blood. At 9 Ringer's solution was replaced by arterial blood, and this at 10 by catheter blood collected at the level of the adrenals. Reduced one-half.

FIG. 15. Uterus tracings. Blood from the same cat used for Fig 14. At 33 Ringer's solution was replaced by catheter blood from the adrenal level, and this at 34 by adrenal blood; at 35 Ringer's solution was replaced by catheter blood, and this at 36 by adrenal blood. At 37 Ringer's solution was replaced by catheter blood collected during the clipping of both adrenals, and this at 38 by adrenalin (1:1,000,000) in the same blood. At 39 Ringer's solution was replaced by catheter blood from the level of the adrenals, and this at 40 by adrenalin (1:3,300,000) in the same blood. At 42 Ringer's solution was replaced by

adrenalin (1:16,500,000) in catheter blood; at 43, by the same catheter blood without the addition of adrenalin; at 45, by another catheter specimen collected at the adrenal level; at 46, by adrenalin (1:65,000,000) in the catheter blood used for Observation 45.

PLATE 53.

FIG. 16. Intestine tracings. Blood from the same cat used for Figs. 14 and 15. At 19 Ringer's solution was replaced by catheter blood collected with the adrenal veins clipped, and this at 20 by catheter blood collected at the adrenal level with the adrenal veins open. At 21 this catheter blood was replaced by arterial blood, and this at 22 by adrenal blood. Reduced one-third.

FIG. 17. Intestine tracings. Bloods from a dog anesthetized with ether. At 7 Ringer's solution was replaced by indifferent blood, and this at 8 by the fifth adrenal specimen. At 23 Ringer's solution was replaced by indifferent blood, and this at 24 by catheter blood, collected at the level of the adrenals during sciatic stimulation. At 25 Ringer's solution was replaced by indifferent blood, and this at 26 by the fifth adrenal specimen, diluted with ten volumes of indifferent blood. At 27, 29, and 31 Ringer's solution was replaced by indifferent blood, and this at 28, 30, and 32 by the fifth adrenal specimen, diluted respectively with twenty, forty, and eighty volumes of indifferent blood. All the bloods were diluted with four volumes of Ringer's solution before application to the segment. Reduced one-third.

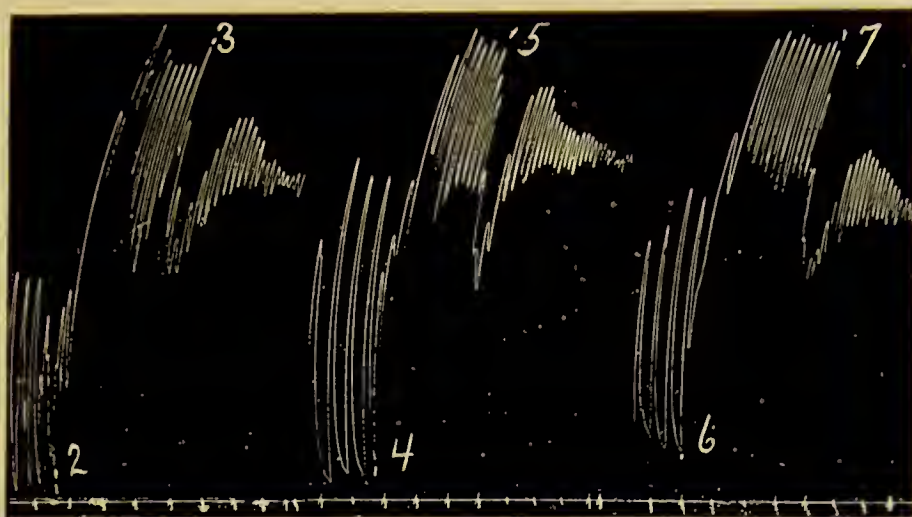


FIG. 1.

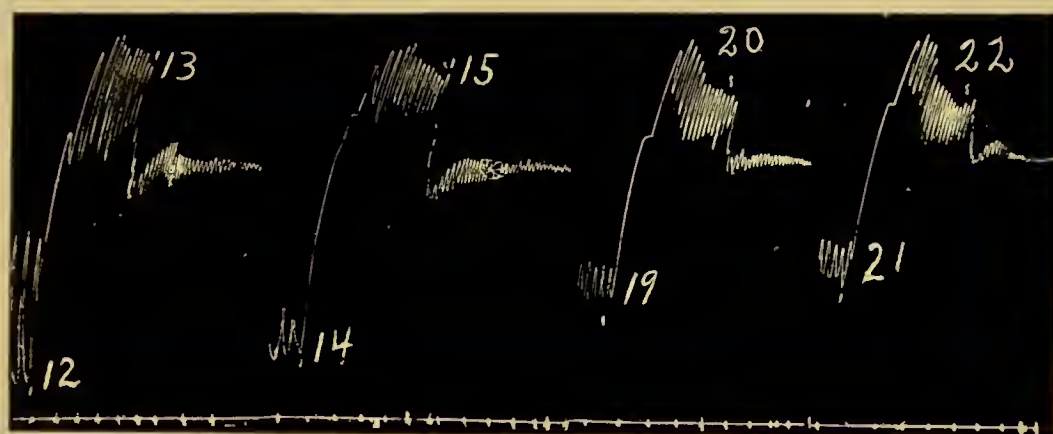


FIG. 2.



FIG. 3.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

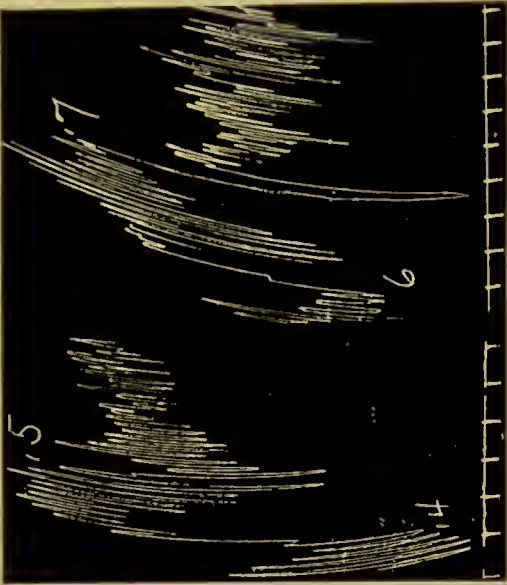


FIG. 5.

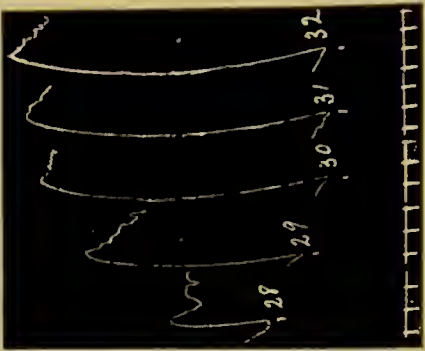


FIG. 7.

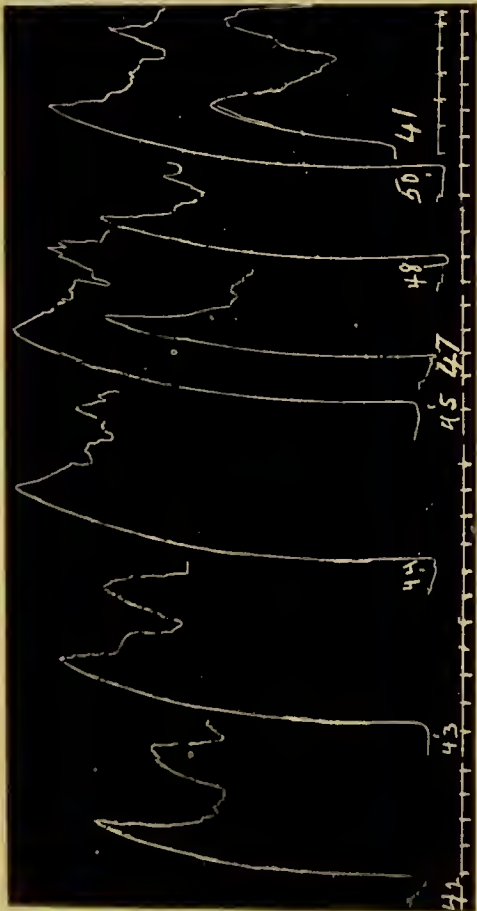


FIG. 4.

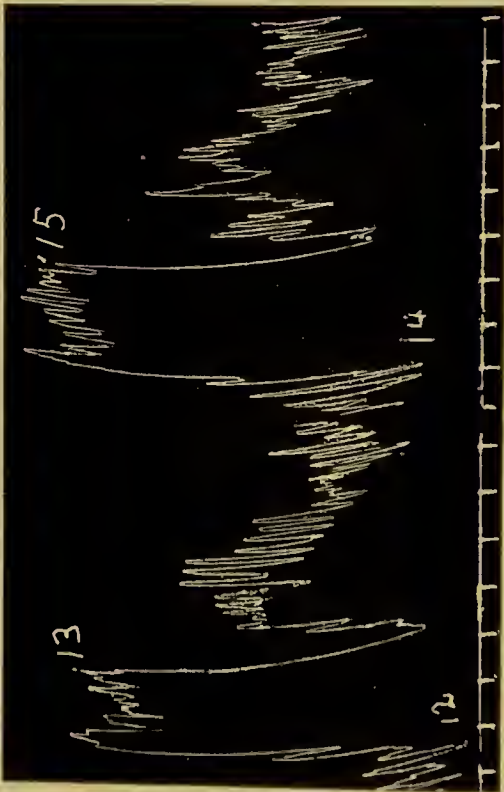


FIG. 6.

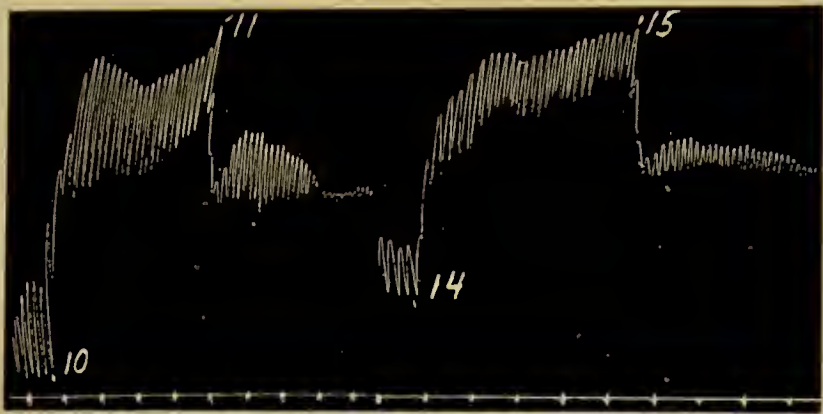


FIG. 8.

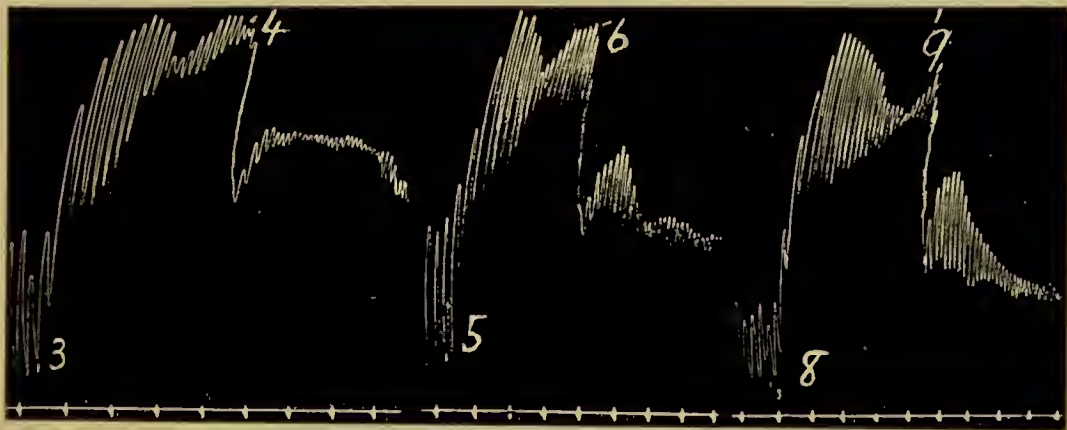


FIG. 9.

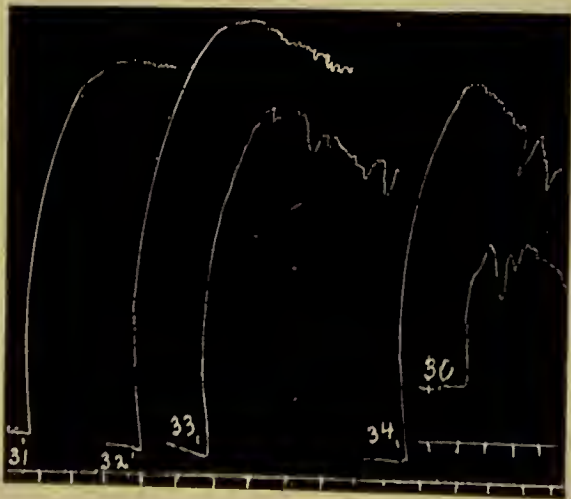


FIG. 10.

(Stewart and Rogoff: Rate of liberation of epinephrin.

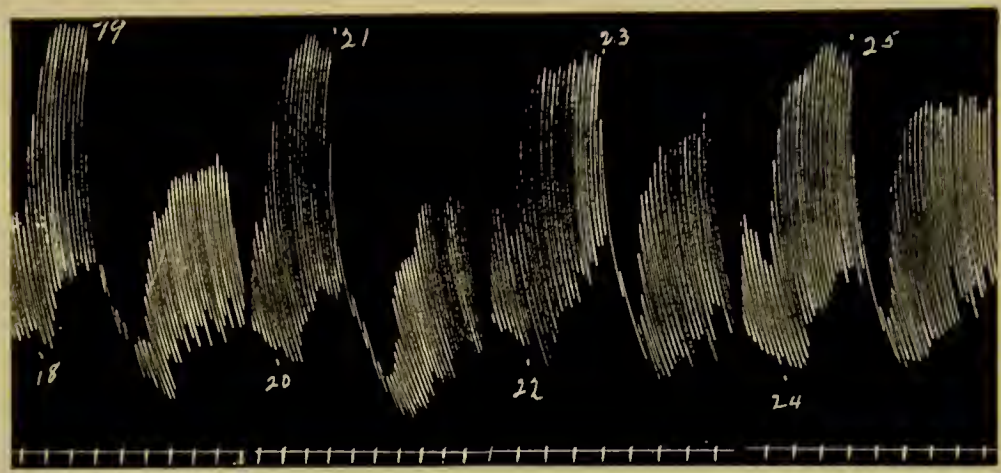


FIG. 11.

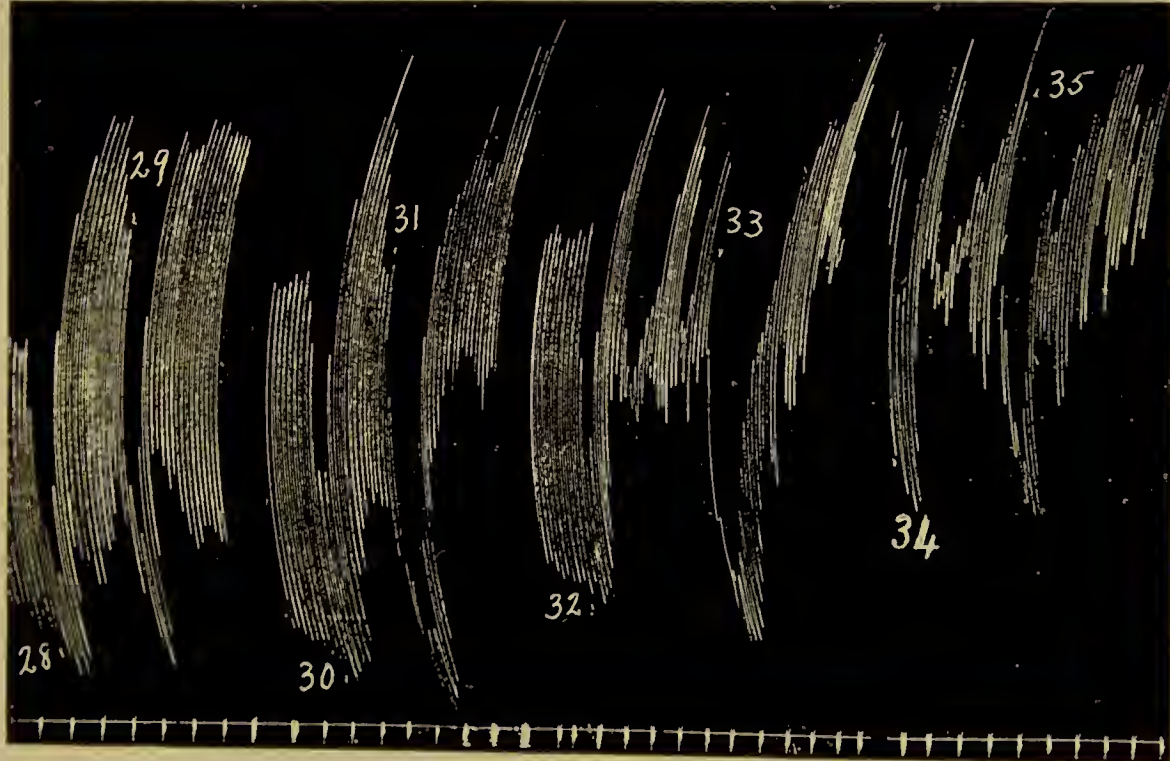


FIG. 12.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

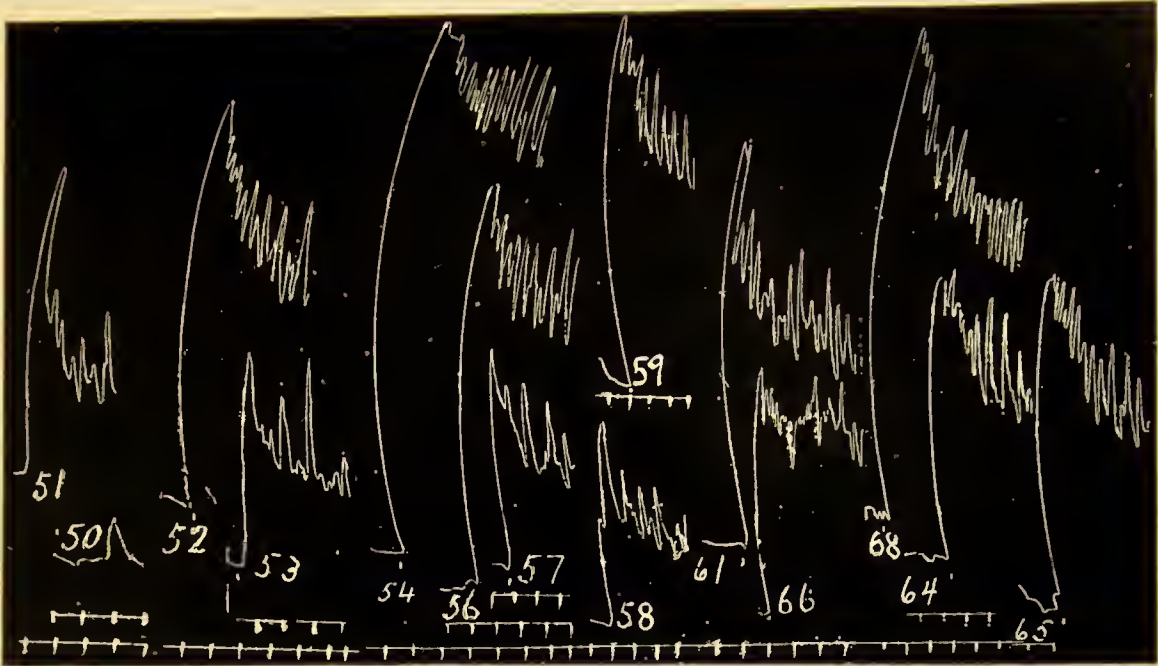


FIG. 13.

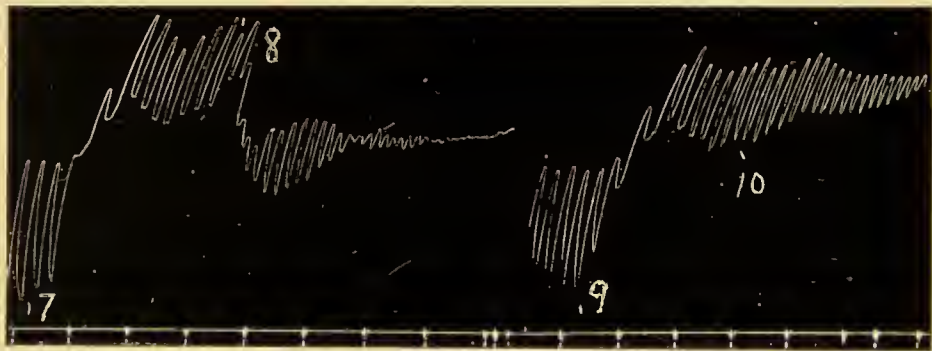


FIG. 14.

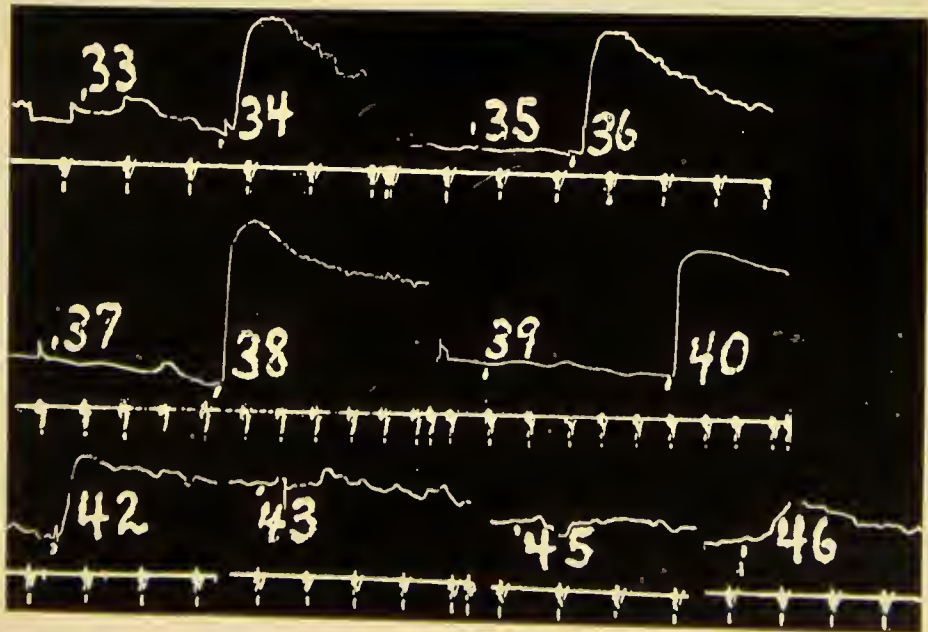


FIG. 15.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

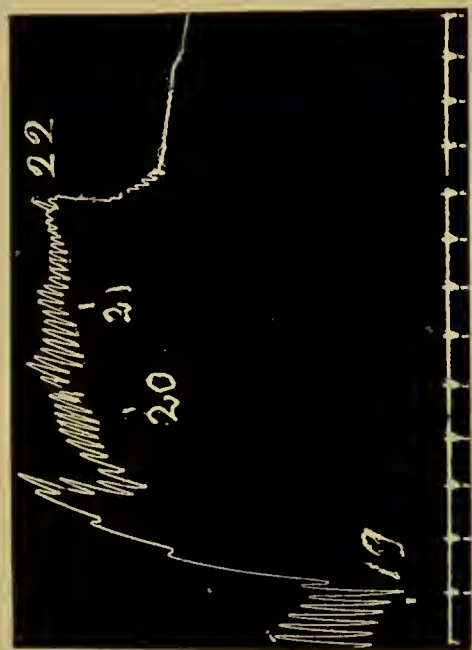


FIG. 16.

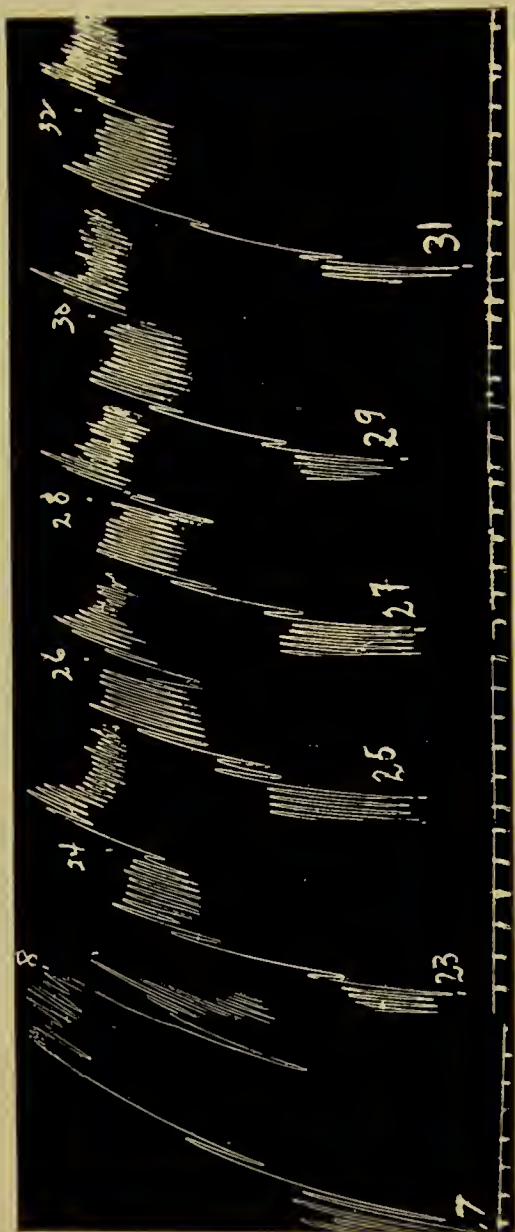


FIG. 17.

(Stewart and Rogoff: Rate of liberation of epinephrin.)

THE RELATION OF THE RATE OF THE SPONTANEOUS LIBERATION OF EPINEPHRIN TO THE RATE OF BLOOD FLOW THROUGH THE ADRENALS

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In several of our papers we have given illustrations of the fact that the rate of liberation of epinephrin (in the cat) may remain approximately constant for a wide range in the rate of blood flow through the adrenals, the concentration in the adrenal vein blood varying inversely as the flow (1). Numerous assays, incidentally accumulated, have indicated not only that this is true in one and the same animal under experimental conditions, but also that the output in different cats varies less than might have been expected (2). It has seemed worth while, however, to supplement these observations by some experiments specially directed to the question whether with such quantitative methods as have been employed an exact inverse ratio between epinephrin concentration and adrenal blood flow can be made out. It will be more instructive to reproduce a sufficient number of the tracings from a typical assay to illustrate fully the degree of sharpness which can be obtained in such observations rather than to give one or two specimens from each of the experiments. We lay stress on making multiple observations on any specimen of blood which is being assayed for epinephrin by the rabbit intestine and uterus segment method. With a single segment a very large number of observations can be carried out without loss of accuracy in the comparisons if two simple precautions be adopted: First, always comparing tracings which are not too far apart in the series; and second, repeating observations freely. It is scarcely necessary to observe that although some of the experimental conditions may be changed at will in the course of a series of observations, for example, the weight, comparison must not be made of tracings taken before with tracings taken after the change. A suitable intestine segment, far from deteriorating from long use, is often seen to improve after a time as regards constancy

of response. This does not mean that it also improves in regard to sensitiveness although segments long worked with may still be very sensitive to small concentrations of epinephrin. An exceedingly sensitive segment, however, although indispensable when the business is to detect minimal quantities of epinephrin, for example, in determining whether any liberation is taking place after section of the nerves of the adrenal (2) is not necessarily advantageous in assaying such concentrations as normally exist in blood coming from the adrenal veins. Here, constancy of response is far more important than sensitiveness. It scarcely needs to be pointed out that a segment may distinguish clearly between blood containing no epinephrin and blood containing 1:100,000,000, and yet may not give a clear difference between two bloods containing respectively 1:3,000,000 and 1:3,500,000 even when the bloods are applied in all degrees of dilution. All the tracings given in figures 1 to 13, as well as others not reproduced, were obtained from one intestine segment, on which ten specimens of blood were assayed.

Experiment 1. Condensed protocol. Cat (pregnant). Weight, 3.03 kgm. Urethane, 5 grams by stomach tube. Inserted tracheal and jugular cannulae and obtained a specimen of jugular blood. Made a "short"¹ cava pocket, tying the renal, coeliac and mesenteric arteries and the abdominal aorta. Then collected the following specimens of adrenal blood.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION	BLOOD FLOW PER MINUTE
	<i>grams</i>	<i>minutes</i>	<i>grams</i>
1	4.7	2	2.3
2	7.8	4	2.0
3	10.1	6	1.7
4	9.8	8	1.2
5	3.5	9	0.4

Now collected more blood from the jugular. Combined weight of the two adrenals 0.360 grams. The bloods were immediately put on ice and after two hours tested on rabbit intestine and uterus segments, an assay of the epinephrin being made on the former.

¹ Where blood is collected from the cava pocket through a cannula it is advantageous to make the pocket much shorter (by inserting the cannula higher up in the vein) than in cases where the pocket is used as a receptacle to accumulate adrenal vein blood which is then released into the circulation. In the latter case it is essential to have a capacious pocket, whereas in the former it is more important to reduce the dead space, so that overlapping of successive samples as well as the risk of clotting may be minimized.

Tracings illustrating the assay of the various adrenal blood samples on a rabbit intestine segment are reproduced in figures 1 to 7. Figure 1 shows that the fourth specimen (observation 32) has a distinctly greater effect on the segment than the second (observation 30); and that the concentration of epinephrin in the fourth specimen is somewhat greater than 1:2,700,000 (observation 36). The adrenalin (Parke, Davis and Company) was assayed by the colorimetric method of Folin, Cannon and Denis. It was shown by the uterus tests (not reproduced)

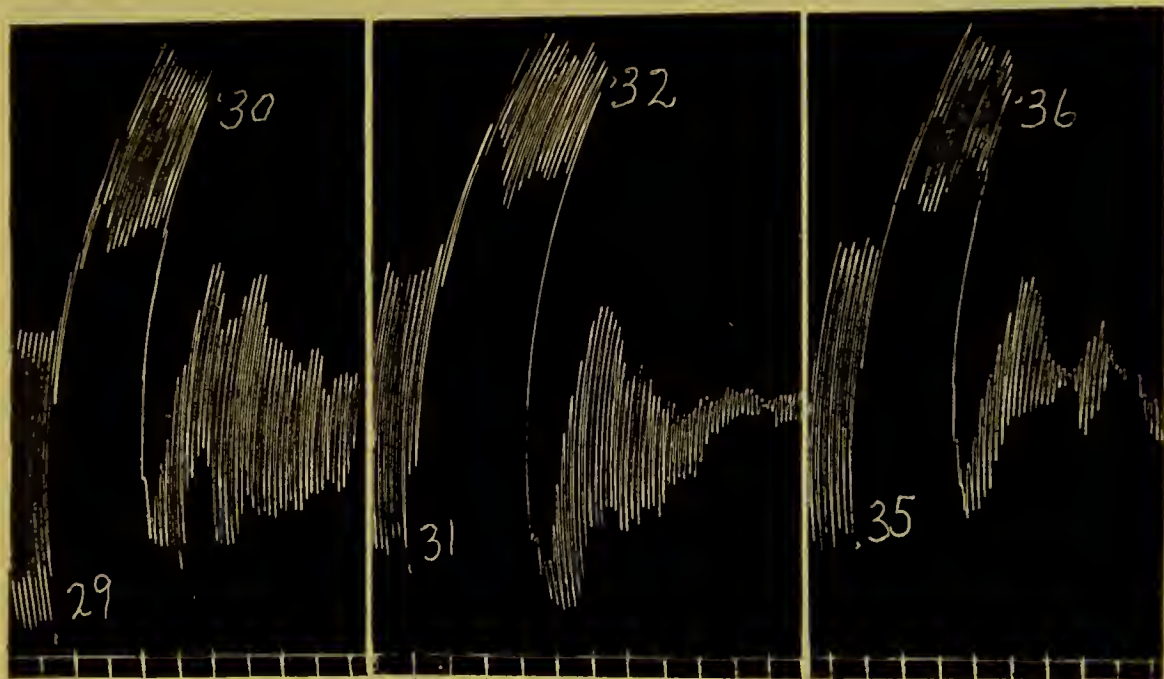


Fig. 1. At 29 and 31 Ringer's solution was replaced by indifferent (jugular) blood, and this at 30 and 32 by the second and fourth adrenal blood specimens, respectively. All the bloods were diluted with an equal volume of Ringer's solution. At 35 Ringer was replaced by jugular blood, and this at 36 by jugular blood to which adrenalin had been added to make up a concentration of 1:2,700,000, the adrenalin blood being then diluted with its own volume of Ringer's solution before application to the segments.²

² Figures 1 to 13 are tracings from a rabbit intestine segment. All have been reduced to two-thirds. The tracings in figures 1, 2, 8 and 9 were taken with a lighter weight than those in the other figures. Figures 1 to 7 are from bloods of one cat (experiment 1); figures 8 to 13 from bloods of another cat (experiment 2). Figure 14 shows tracings from a rabbit uterus segment, reduced to one-half, with blood from a dog (experiment 3). Figures 15 to 17 are rabbit intestine tracings with bloods from a dog (experiment 5), reduced to two-thirds. In all the tracings time was marked in half-minutes.

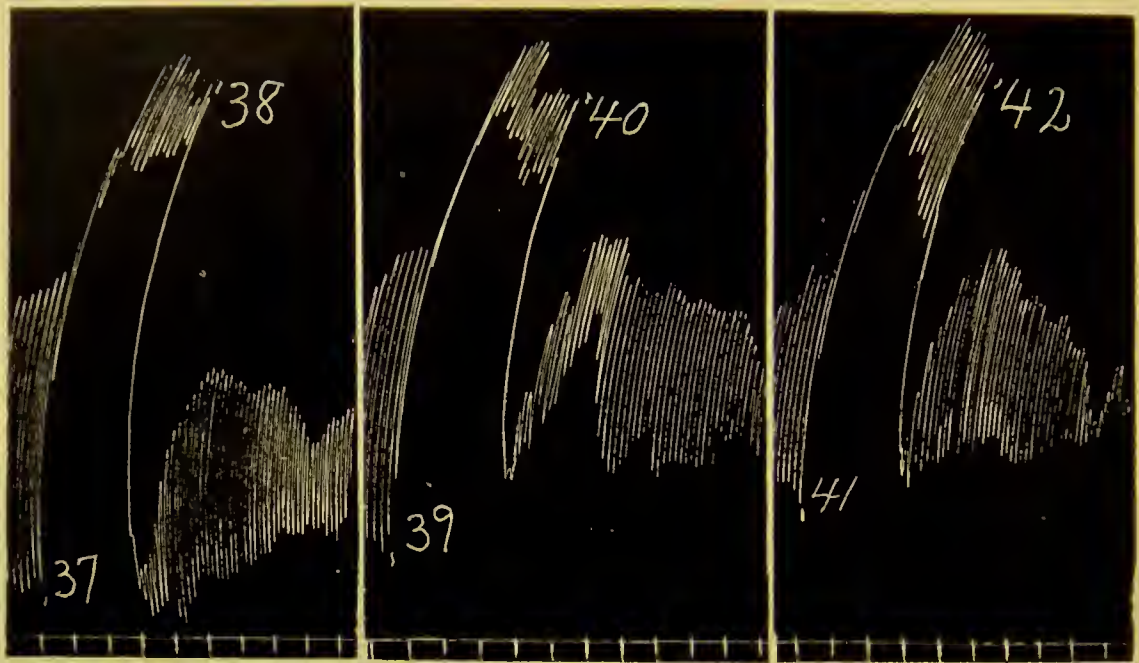


Fig. 2. At 37 and 39 Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer's solution. At 38 and 40 this was replaced by jugular blood made up with adrenalin to a concentration of 1: 2,000,000 and 1: 3,400,000, respectively, the adrenalin blood being diluted with an equal volume of Ringer's solution before application to the segment. At 41 Ringer's solution was replaced by jugular blood, and this at 42 by the second adrenal blood specimen, both bloods being diluted with an equal volume of Ringer.

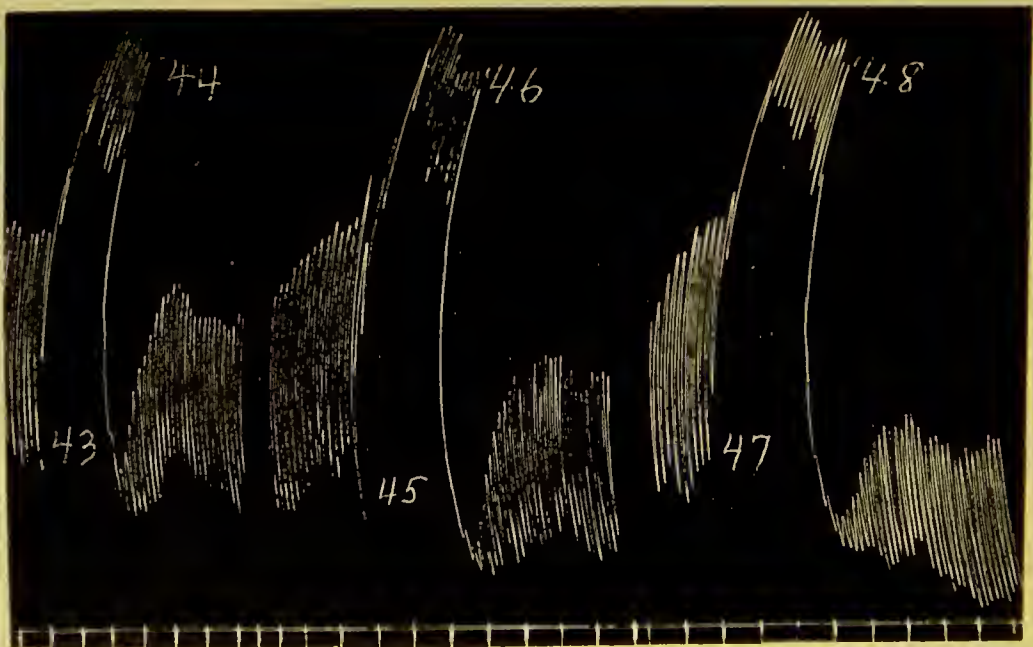


Fig. 3. At 43, 45 and 47 Ringer's solution was replaced by jugular blood, and this at 44, 46 and 48 by the first, third and fourth adrenal specimens, respectively. All the bloods were diluted with an equal volume of Ringer's solution before application to the segment.

that the inhibitory effect of the blood on the intestine was due to a substance which caused an increase of tone of the virgin rabbit uterus, that is, to epinephrin.

In figure 2 it is indicated that the second adrenal specimen (observation 42) has a somewhat greater concentration of epinephrin than 1:3,400,000 (observation 40); but decidedly less than 1:2,000,000 (observation 38), which, however, is not much different from the

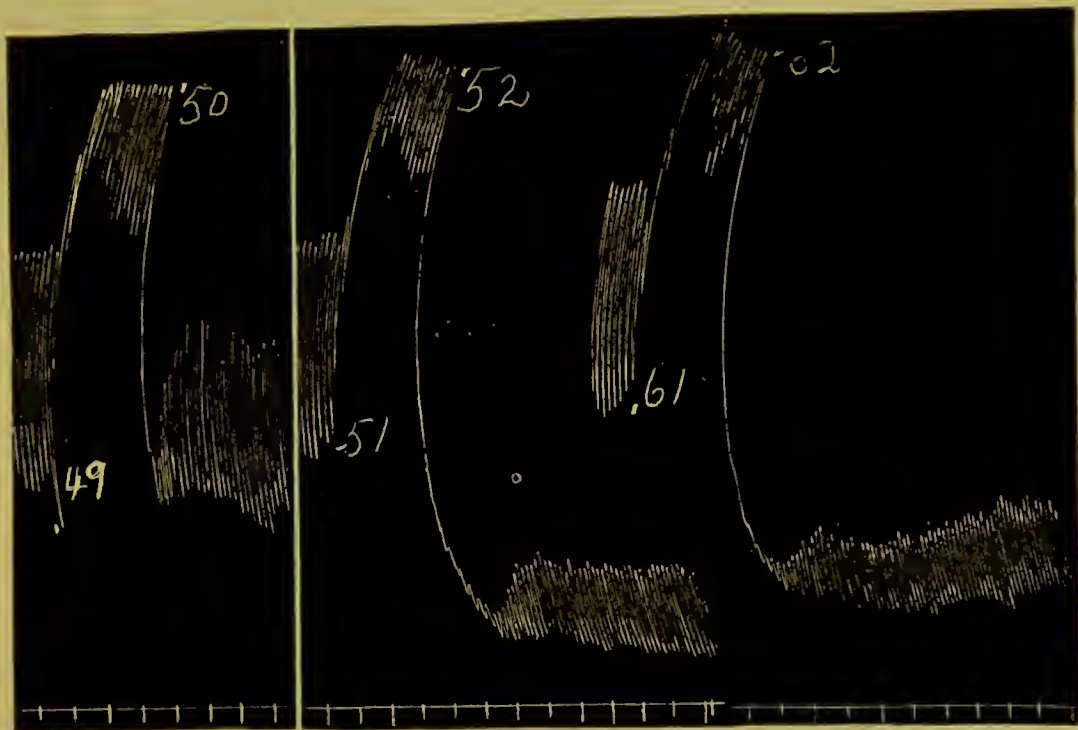


Fig. 4. At 49 and 51 Ringer's solution was replaced by jugular blood, and this at 50 and 52 by the second and the fifth adrenal blood specimens, respectively. All the bloods were diluted with an equal volume of Ringer. At 61 Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer, and this at 62 by jugular blood made up with adrenalin to a concentration of 1:1,350,000, the mixture being then diluted with an equal volume of Ringer's solution before application to the segment.

concentration in the fourth specimen (observation 32, fig. 1). Figure 3 clearly indicates the progressive increase in epinephrin concentration in the first (observation 44), third (observation 46) and fourth specimens (observation 48). Comparison of figure 4 with figure 3 shows that the second specimen (observation 50) does not differ much from the first (observation 44). It must be remembered that the first specimen will contain the epinephrin, if any, liberated by the manip-

ulation incidental to clipping off the upper end of the cava pocket before beginning the collection of blood. On the other hand, a little indifferent cava blood may be included in the pocket when it is clipped off, and this would dilute the first specimen. The result of the tests on the first adrenal specimen shows that in this experiment little epinephrin was liberated by manipulation. The fifth specimen (observation 52, fig. 4) has a much greater concentration of epinephrin than any of the others, and distinctly greater than 1:1,350,000 (observation 62). No estimate can be formed of the magnitude of the difference between the concentration in the fifth specimen and 1:1,350,000

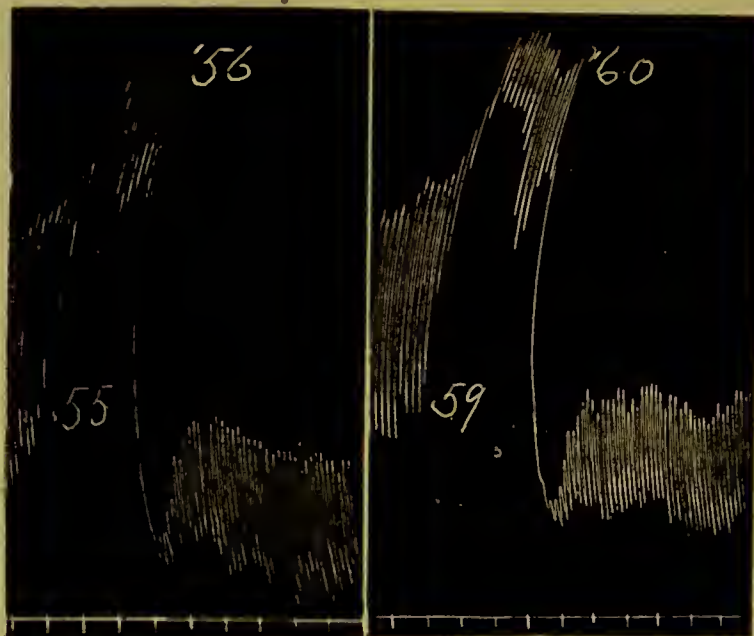


Fig. 5. At 55 and 59 Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer, and this at 56 and 60 by jugular blood made up with adrenalin to a concentration of 1:2,000,000 and 1:2,700,000, respectively; the adrenalin bloods being then diluted with an equal volume of Ringer before application to the segment.

from these two observations, because the inhibitory effect on the intestine segment approaches the maximum effect too nearly. To sharpen the assay greater dilutions of the blood specimen were used later on in the experiment.

Figure 5 indicates that adrenalin in indifferent blood in a concentration of 1:2,000,000 (observation 56), gives practically the same inhibition as the fourth adrenal blood specimen similarly diluted (observation 48, fig. 3). Observation 60 (fig. 5) shows that a con-

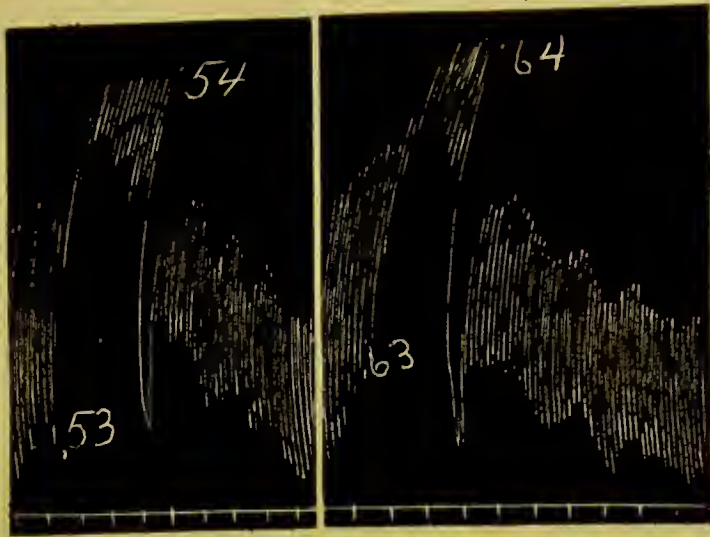


Fig. 6. At 53 Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer and this at 54 by jugular blood made up with adrenalin to a concentration of 1: 3,400,000, the adrenalin blood being then diluted with its own volume of Ringer. At 63 Ringer's solution was replaced by jugular blood and this at 64 by the first adrenal blood sample, both bloods being diluted with an equal volume of Ringer.

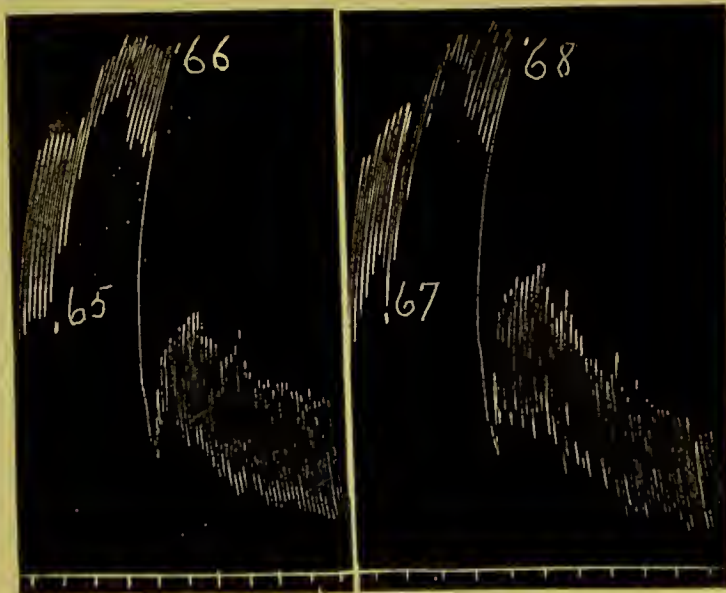


Fig. 7. At 65 and 67 Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer. At 68 this was replaced by the fourth adrenal specimen similarly diluted. At 66 the diluted jugular blood was replaced by a mixture containing one part of the fifth adrenal blood, one part of jugular blood and two parts of Ringer's solution. The ultimate dilution of the epinephrin in the fifth specimen was thus twice as great as in the fourth specimen at observation 68.

centration of 1:2,700,000 is quite distinctly less than that of the fourth specimen (observation 48, fig. 3), but probably somewhat greater than that of the third specimen (observation 46, fig. 3). In figure 6 it is seen that the first adrenal blood sample (observation 64) has a concentration of epinephrin little different from 1:3,400,000 (observation 54), but probably slightly greater. In figure 7 the fourth specimen was compared with the fifth in greater dilution than in figures 3 and 4. Observation 68 shows the effect of the fifth specimen when the epinephrin in it was diluted twice as much as the epinephrin in the fourth specimen (observation 66). The dilution was made in such a way (see legend of figure) that the concentration of blood was the same in the two observations. It is obvious that the curve in observation 68 falls more sharply after the first abrupt drop than that in observation 66, so that in three minutes it has more nearly approached the base line. There is no doubt, then, that the fifth specimen contains more than twice as much epinephrin as the fourth. It should have three times as great a concentration if the rate of liberation was constant during this period of the experiment, since the rate of blood flow during collection of the fifth sample was only one-third of that during collection of the fourth. It is necessary, however, to point out that as concentrations much greater than 1:1,000,000 are not found in the adrenal vein blood of cats, at least as collected under our experimental conditions and assayed on rabbit segments, the strict inverse ratio of concentration and blood flow is bound to fail for samples collected in the neighborhood of the maximum possible concentration. If we compare the second and fourth specimens, leaving out the first for the reason mentioned, although it certainly does not differ much from the second, we obtain between the concentrations (1:2,000,000 for the fourth, \approx 1:3,400,000 for the second) a ratio of 1:1.7. The ratio between the blood flows is also 1:1.7. Taking the concentration of the third specimen as 1:2,700,000, the ratio between the concentrations of the third and fourth specimens is 1:1.4. The ratio between the blood flows of the fourth and third specimens is also 1:1.4.

Taking the concentration of the second specimen as 1:3,400,000 and that of the third as 1:2,700,000, the ratio between the concentrations is 1:1.3. The ratio between the blood flows of the two specimens is 1:1.2. It would be absurd to refine on such calculations, considering the degree of accuracy possible in epinephrin assays even under the best conditions with such small concentrations as are present in blood. But it is clear enough that in this experiment, for a considerable range of blood flow, the rate of liberation of epinephrin must be assumed to

Experiment 2. Protocol. Cat (pregnant). Weight, 2.975 kgm. Urethane, 4.5 grams. Tracheal and jugular cannulae inserted, and a small sample of jugular blood obtained. Then a short cava pocket was made with ligation of renal arteries and abdominal aorta, and the following specimens of adrenal blood collected.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION	BLOOD FLOW PER MINUTE
	<i>grams</i>	<i>minutes</i>	<i>grams</i>
1	5.2	2	2.6
2	9.3	4	2.3
3	11.6	5	2.3
4	10.6	5	2.1
5	11.1	6	1.8
6	5.4	10	0.5

The sixth specimen was collected while additional blood was being obtained from the jugular (40 cc.). Combined weight of adrenals, 0.58 gram.

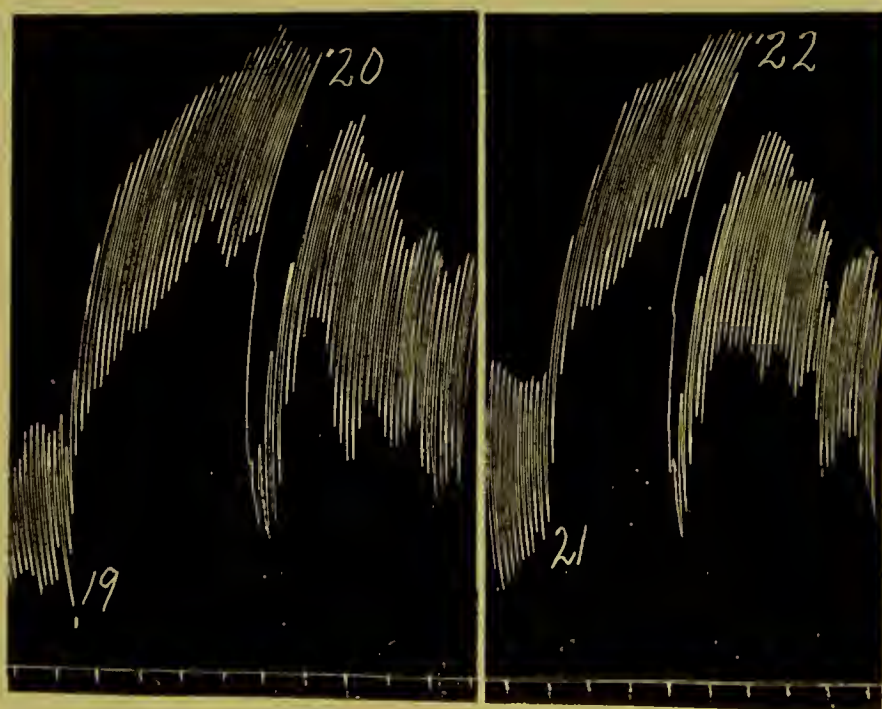


Fig. 8. At 19 Ringer's solution was replaced by indifferent (jugular) blood diluted with an equal volume of Ringer, and this at 20 by the second adrenal blood sample (from experiment 2) similarly diluted. At 21 Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer, and this at 22 by jugular blood made up with adrenalin to a concentration of 1:4,000,000, the adrenalin blood being then diluted with its own volume of Ringer before application to the segment. The weight used was less than in the other figures from experiment 2 except figure 9.

have remained approximately constant. It necessarily follows that the concentration of epinephrin in the blood of the adrenal veins must have varied inversely as the blood flow through the glands.

The same thing is demonstrated fully as well in experiment 2. The initial concentration in this experiment being smaller than in the previous one, the rule of the inverse ratio applies even to the specimen collected with the slowest flow.

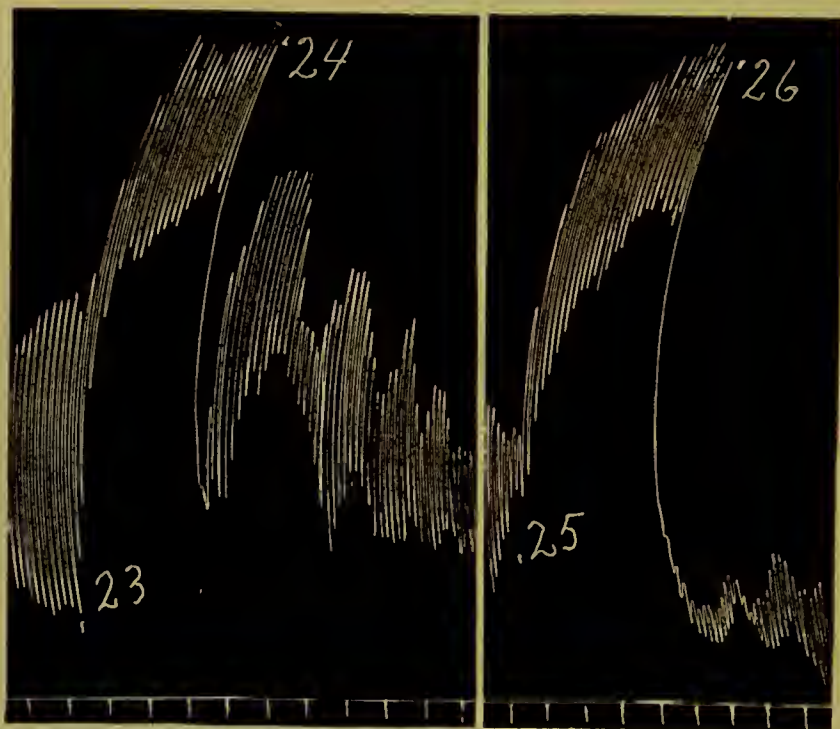


Fig. 9. At 23 and 25 Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer, and this at 24 and 26 by jugular blood made up with adrenalin to concentrations of 1:2,700,000 and 1:1,350,000 respectively; the adrenalin bloods being then diluted with an equal volume of Ringer's solution before application to the segment. The weight used was less than in the other figures from experiment 2 except figure 8.

The bloods from experiment 2 were tested on the same intestine segment as those from experiment 1. Figure 8 shows that the second adrenal blood specimen from experiment 2 (observation 20) produces an effect on the intestine nearly the same as that produced by indifferent (jugular) blood containing adrenalin in the concentration of 1:4,000,000. The epinephrin concentration in this specimen was shown to be decidedly less than 1:2,700,000, and very much less than 1:1,350,000 (fig. 9, observations 24 and 26). These curves are reproduced to prove that

the sensitiveness of the segment to different concentrations of adrenalin in blood was sufficient for the purposes of the assay. Figures 10 and 11 display the effects of all the adrenal blood specimens from experiment 2 on the intestine segment. The second and third specimens (observations 72 and 74, fig. 10) produced practically the same inhibition, and it will be seen from the protocol that during their collection the blood flow remained unchanged.

These tracings cannot be quantitatively compared with those reproduced in figures 8 and 9, because the weight attached to the lever

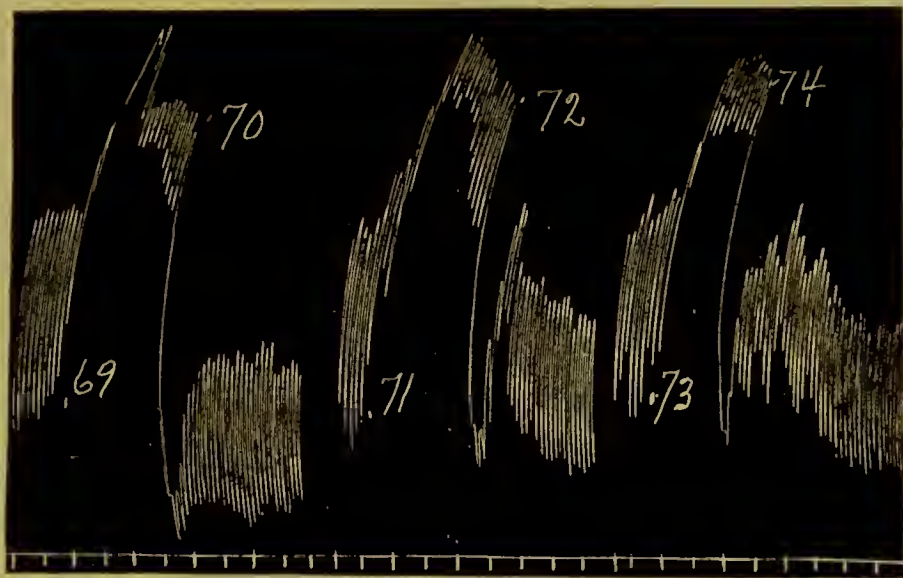


Fig. 10. At 69, 71 and 73 Ringer's solution was replaced by jugular blood and this at 70, 72 and 74, respectively, by the first, second and third adrenal blood specimens (experiment 2). All the bloods were diluted with an equal volume of Ringer's solution.

had been increased after observation 42 (fig. 2). Also, as already mentioned, it is a rule in this method of assaying only to compare observations not very far apart in the series. The somewhat greater inhibitory effect caused by the first specimen (observation 70, fig. 10) than by the second or third is doubtless due to some slight "manipulative" liberation of epinephrin while the pocket was being closed off. Observation 80 (fig. 11) indicates a slightly greater concentration for the fourth adrenal sample than for the third (observation 74, fig. 10), and the rate of blood flow during collection of the fourth specimen was slightly less than during collection of the third. Observation 82 (fig. 11) suggests that the epinephrin concentration in the fifth specimen is

somewhat greater than in the fourth. In particular, the transient rise following the first abrupt drop is less than in observation 80. The blood flow during collection of the fifth specimen was somewhat less than during collection of the fourth. The inhibition produced by the sixth adrenal blood sample (observation 84, fig. 11) obviously corresponds to a very much greater epinephrin concentration than that of any of the other specimens, and the protocol shows that the blood

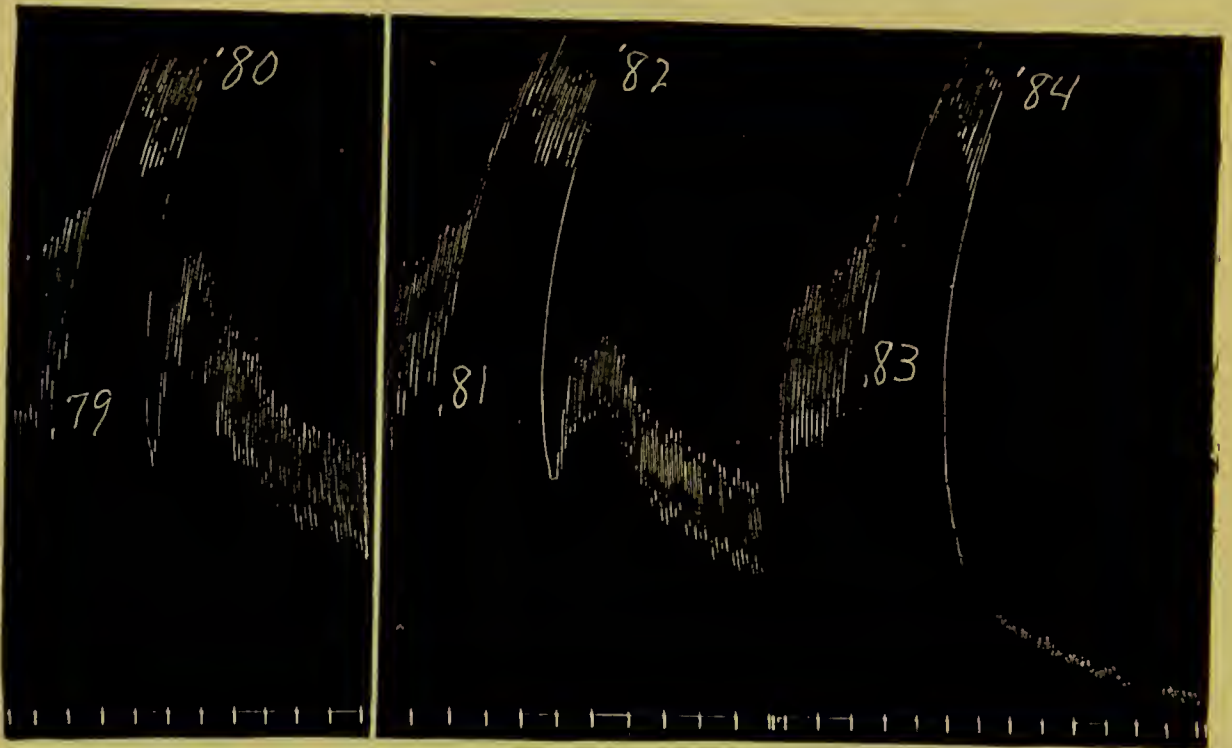


Fig. 11. At 79, 81 and 83 Ringer's solution was replaced by jugular blood, and this at 80, 82 and 84, respectively, by the fourth, fifth and sixth adrenal blood specimens (experiment 2). All the bloods were diluted with one volume of Ringer's solution. The drum was lowered in the interval between figures 10 and 11, so as to obtain space for the drop expected in observation 84. The greater distance of the curves from the base line accordingly does not indicate that the tone of the segment beating in Ringer's solution had increased.

flow during collection of the sixth specimen was by far the smallest of all. The observations illustrated in figures 10 and 11 are sufficient to show qualitatively an inverse relation between the rate of blood flow and the epinephrin concentration. Figures 12 and 13 complete the quantitative proof. Observation 86 (fig. 12) indicates that 1:2,700,000 is a little greater than the concentration in the fifth specimen (observation 82, fig. 11). The initial drop in observation 86 is somewhat greater,

and the subsequent transient recovery of tone decidedly less than in observation 82. It was previously determined that the concentration in the second specimen of experiment 2 was approximately 1:4,000,000. If the concentration in the fifth specimen be taken even as 1:2,700,000, and it is less than this, the ratio between the concentrations of the second and fifth specimen is 1:1.5, whereas the ratio between the blood flows of the fifth and second specimens is 1:1.3. If the concentration of the fifth specimen be taken as 1:3,000,000, the ratio between the

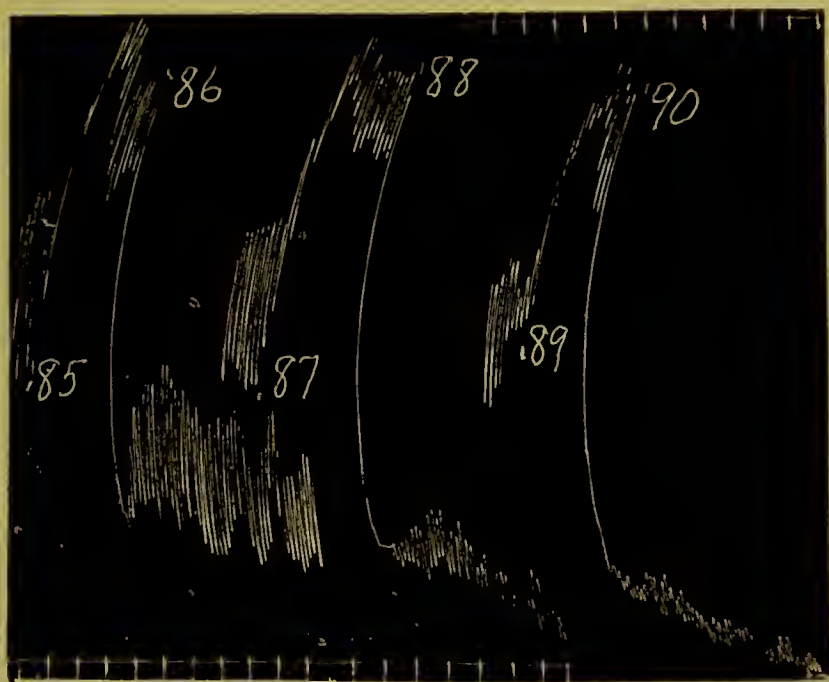


Fig. 12. At 85, 87 and 89 Ringer's solution was replaced by jugular blood diluted with an equal volume of Ringer, and this at 86, 88 and 90 by jugular blood made up with adrenalin to concentrations of 1:2,700,000, 1:1,350,000 and 1:700,000 respectively, the adrenalin bloods being then diluted with an equal volume of Ringer before application to the segment.

concentrations will also be 1:1.3. Comparing observation 84 (fig. 11) with observations 88 and 90 (fig. 12). it is clear that the concentration in the sixth specimen is superior to 1:1,350,000, and not very different from 1:700,000. The response of the intestinal segment to such high concentrations of epinephrin is, however, too near the maximum response for the greatest accuracy of which the method is capable. Accordingly observations were made with a greater degree of dilution.

In figure 13 it is shown that the concentration of epinephrin in the sixth specimen (observation 92) is somewhat less than 1:700,000 (ob-

servation 94), and decidedly greater than 1:950,000 (observations 98 and 100). If the concentration in the sixth specimen be taken as 1:800,000 and that in the fifth specimen as 1:3,000,000, the ratio between the concentrations of the fifth and sixth samples comes out 1:3.7. Taking the concentration in the fifth specimen as 1:2,700,000, the ratio would be 1:3.4. The ratio between the blood flows for the sixth and fifth specimens is 1:3.6. In this experiment, with a range of blood flow from 2.3 grams per minute to 0.5 gram per minute, the inverse ratio between blood flow and epinephrin concentration in the adrenal blood was maintained; in other words, the rate of liberation of epi-

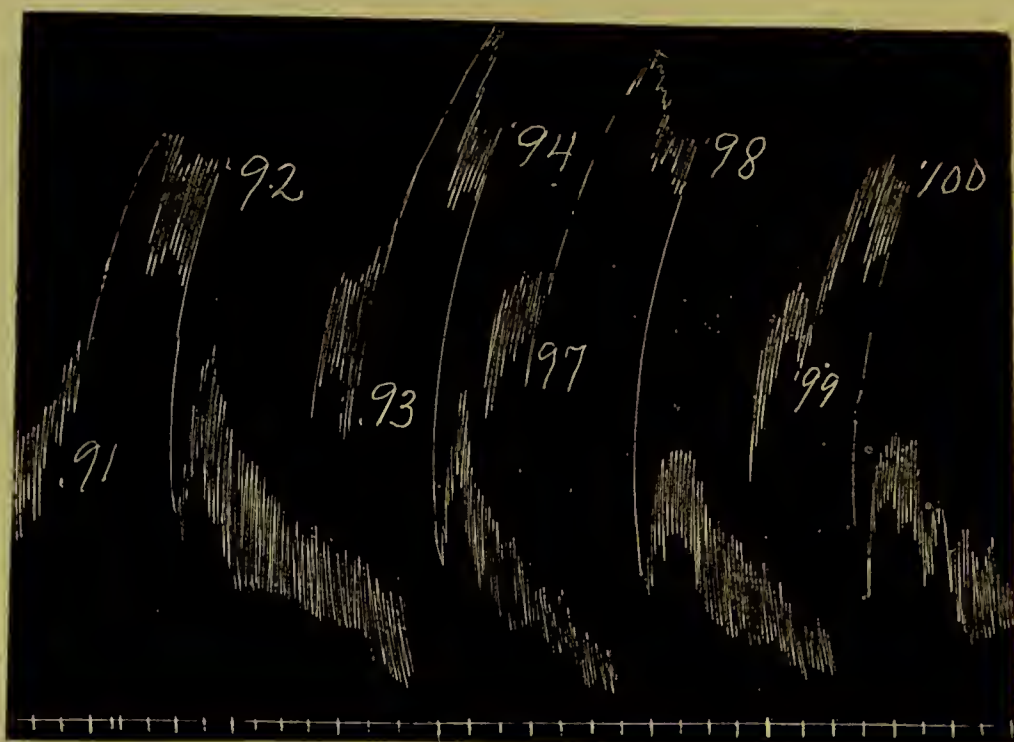


Fig. 13. At 91 Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer. At 92 this was replaced by a mixture containing one part of the sixth adrenal blood, (from experiment 2) one part of jugular blood and two parts of Ringer's solution. At 93 Ringer's solution was replaced by jugular blood diluted with its own volume of Ringer, and this at 94 by a mixture containing one part of jugular blood made up with adrenalin to a concentration of 1:700,000, one part of jugular blood and two parts of Ringer. At 97 Ringer's solution was replaced by jugular blood, diluted with two volumes of Ringer, and this at 98 by the sixth adrenal specimen (from experiment 2) similarly diluted. At 99 Ringer's solution was replaced by jugular blood diluted with two volumes of Ringer, and this at 100 by jugular blood made up with adrenalin to a concentration of 1:950,000, the adrenalin blood being then diluted with two volumes of Ringer before application to the segment.

nephrin remained constant for the whole of this experimental period. It must be assumed that in this animal even when the very high concentration of 1:800,000 had been reached in the blood, the glands were still able to pass into the blood the full amount of epinephrin delivered per unit of time with greater blood flows. Since, however, 1:800,000 represents a concentration scarcely ever surpassed in adrenal blood, collected and assayed in the manner described, it may be concluded with confidence that had collection of the adrenal blood been continued with still smaller rates of flow, the inverse proportionality would of necessity have disappeared and the amount of epinephrin liberated per minute into the blood would have shown a progressive diminution.

In general it may be said that the approximate constancy of the rate of epinephrin liberation under the experimental conditions can be demonstrated throughout the greatest range of blood flow when the initial concentration is low, or what usually although not invariably comes to the same thing, when the initial blood flow is high. When the concentration in the first samples is already high, the rate of liberation will soon appear to diminish as the rate of blood flow falls below the point corresponding to the maximum possible concentration. A few more results illustrating this rule may be quoted in summarized form.

In a cat weighing 2.035 kgm., the protocol of which has been published elsewhere (3), the second adrenal blood specimen, with a flow of 2.2 grams per minute, had a concentration of about 1:2,500,000 of epinephrin; and the third adrenal specimen, with a flow of 1.3 gram per minute, a concentration of about 1:1,500,000. The ratio of the concentrations of the second and third specimens is 1:1.7, and the ratio of the blood flows of the third and second specimens is also 1:1.7. The output of epinephrin was therefore unchanged during collection of the two samples (0.0009 mgm. per minute). This is a relatively large output as assayed in drawn blood on rabbit segments. In the fifth adrenal specimen, the output calculated from the epinephrin assay was much less, 0.0004 mgm. per minute. But this was inevitable, since the blood flow was only 0.4 gram per minute, and the concentration which was already approaching the maximum limit during collection of the third specimen, could not increase in proportion to the decrease of blood flow, although it reached the high value of 1:1,000,000.

In another cat, weighing 2.66 kgm. (2), the concentration in the second adrenal sample was 1:4,500,000, and in the fifth, 1:2,300,000. The blood flows were 2.1 grams and 1.0 gram per minute, respectively. The ratio between the concentrations of the second and fifth specimens

is 1:2, and that between the blood flows of the fifth and second, 1:2.1. The output of epinephrin per minute was therefore practically the same for the two samples, about 0.00045 mgm. per minute.

In another cat, weighing 3.16 kgm., the concentration in the second adrenal specimen was 1:1,700,000, with a blood flow of 1.30 gram per minute, and in the third specimen, 1:1,000,000, with a flow of 0.73 gram per minute. The rate of liberation of epinephrin was relatively large in this animal, and therefore the maximum concentration was reached with a greater blood flow than would have been the case had the output been smaller. The ratio between the concentrations in the two specimens is 1:1.7, and the ratio between the blood flows, 1:1.8. The output of epinephrin per minute was practically the same for the two samples, about 0.00075 mgm.

In a cat weighing 2.435 kgm. the concentration of epinephrin was found the same, namely 1:2,000,000, in the second, third and fourth adrenal blood specimens, and the blood flows were also equal (1.3 gram per minute). The output of epinephrin per minute was 0.0006 mgm.

In a cat weighing 2.31 kgm., the unusually low concentration of 1:13,000,000 of epinephrin was found in the second adrenal blood specimen, associated with the unusually high blood flow of 5.0 gram per minute (4). In the eighth adrenal specimen, after section of the cord in the cervical region, the concentration mounted to something less than 1:800,000, as great a concentration of epinephrin as we have ever seen in the drawn adrenal blood of a cat. The blood flow during collection of the sample was only 0.3 gram per minute. The concentration in the eighth specimen was accordingly about sixteen times as great as in the second, and the rate of blood flow during collection of the eighth specimen was sixteen times smaller than during collection of the second. The output of epinephrin therefore remained unaltered, namely, 0.0004 mgm. per minute.

In dogs the available data are less extensive but similar results have been obtained. In an experiment on a dog weighing 14.9 kgm., the protocol of which has been published in another connection (4), the concentration of epinephrin in the fifth adrenal blood specimen was about 1:3,000,000; and in the eighth specimen, about 1:1,100,000. The blood flows were 5.7 cc. and 1.8 cc. per minute. The rate of liberation of epinephrin per minute was therefore practically the same in the two samples, notwithstanding the great difference in the blood flow. No doubt the correspondence would have been still more exact had the

concentration in the final sample not been approaching so near the maximum.

In some of the dogs the attempt was made to vary the blood flow through the adrenals by causing incomplete inhibition of the heart by vagus stimulation. However, the effect, was not very decided. The rule abundantly illustrated in the experiments on cats, that a specimen of adrenal blood collected with a slower flow has a greater concentration of epinephrin than one collected in the same experiment with a faster flow, was verified in every case in the observations on dogs. So many intestine tracings having been already reproduced, it will be sufficient to give a sample of uterus tracings (fig. 14, experiment 3) illustrating this point.

Experiment 3. Protoeol. Dog (female). Weight, 6.7 kgm. Ether. Cava pocket prepared with cannula in right iliac vein. One vagus prepared for stimulation in the neck. The following samples of blood were then collected:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
		minutes	seconds		
1	28.0	1	45	16.0	
2	31.0	1	50	16.9	
3	42.0	2	20	18.2	Vagus stimulation
4	30.5	2	45	11.9	Vagus stimulation
5	20.0	2	10	9.2	
6	19.5	3		6.5	Vagus stimulation
7	20.0	4	45	4.2	
8	18.0	7		2.6	
9	8.7	6	30	1.3	

Blood was now collected from the carotid artery and used as the indifferent blood in testing with rabbit intestine and uterus segments. As usual, all bloods were well shaken up with air before being applied to the segments. The combined weight of the adrenals was 0.65 gram.

From the amount of the earlier blood flows, it seems possible that some leakage from other sources than the adrenal veins may have taken place into the pocket, although careful search at autopsy failed to reveal any untied small vein. It must be remembered, however, that with so many arteries clamped the arterial blood pressure is very high at first, and a large blood flow through the adrenals would therefore be expected. In any case, the relative concentration of epinephrin in the successive adrenal samples would probably not be affected, even if a small vein had been overlooked.

The specimens of uterus tracings reproduced in figure 14 suggest that the second adrenal sample (observation 81) has a slightly higher concentration of epinephrin than the third sample (observation 75), corresponding to the slightly greater flow during collection of the latter, notwithstanding the vagus stimulation. The fourth sample (observation 76) has a distinctly greater tone-increasing effect than the third; the fifth sample (observation 77) has a greater effect than the fourth, the sixth (observation 78) a greater effect than the fifth, and the seventh (observation 79) a greater effect than the sixth. The increase of tone produced by the seventh sample was shown to be maxi-

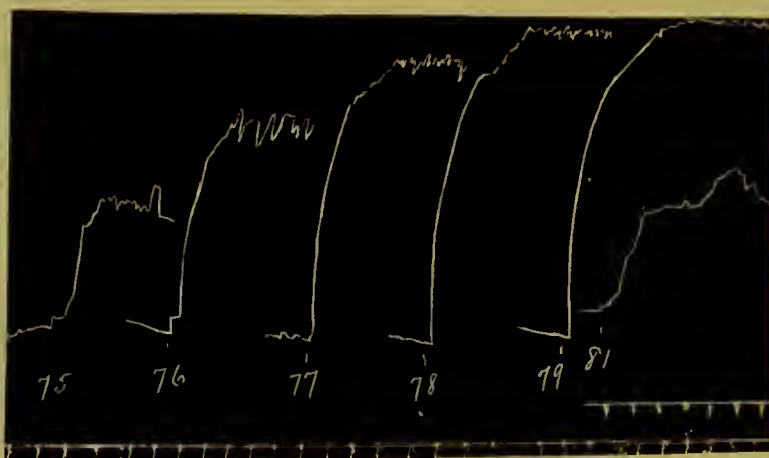


Fig. 14. Uterus tracings. Dog's blood (experiment 3). At 75 Ringer's solution was replaced by the third adrenal blood specimen; at 76 by the fourth; at 77 by the fifth; at 78 by the sixth; at 79 by the seventh; at 81 by the second adrenal sample.

mal for the segment, and that is the reason for the relatively small additional increase caused by the seventh specimen. The eighth and ninth samples naturally produced no further increase of tone. But when the specimens were appropriately diluted a markedly greater effect was caused by the seventh than by the sixth, by the eighth than by the seventh, and by the ninth than by the eighth. Corresponding results were obtained on the intestine segments.

In another dog (experiment 4), a similar experiment was performed and with a similar result.

The epinephrin content of the second adrenal specimen was assayed on a rabbit intestine segment at 1:6,000,000, corresponding to an output of 0.001 mgm. of epinephrin per minute for the animal (0.00015 mgm. per kilogram of body weight per minute). The epinephrin con-

tent of the sixth adrenal specimen was assayed at somewhat more than 1:3,000,000, corresponding to an output of more than 0.0009 mgm. per minute for the animal, practically the same as for the second specimen. The concentrations in the two specimens were therefore approximately in the inverse ratio of the blood flows. Several additional experiments were made on dogs, of which only one more will be cited.

Experiment 4. Protocol. Dog (female in early pregnancy). Weight, 7.7 kgm. Ether. Cava pocket made. Renal and iliac arteries and left iliac vein tied. Cannula inserted into right iliac vein. Left vagus cut in the neck and its peripheral end prepared for stimulation. The following specimens of adrenal blood were then collected.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	cc.	minutes	seconds	cc.	
1	13.2	1	40	8.0	
2	13.0	2		6.5	Vagus stimulation
3	12.5	2	30	5.0	Vagus stimulation
4	16.6	4	28	3.7	
5	12.6	4	29	2.8	
6	7.0	2	30	2.8	Vagus stimulation

A sample of jugular vein blood (17 cc.) was drawn before collection was begun from the cava pocket. After collection of the adrenal samples, 120 cc. of blood was obtained from the carotid artery. The combined weight of the adrenals was 0.78 gram.

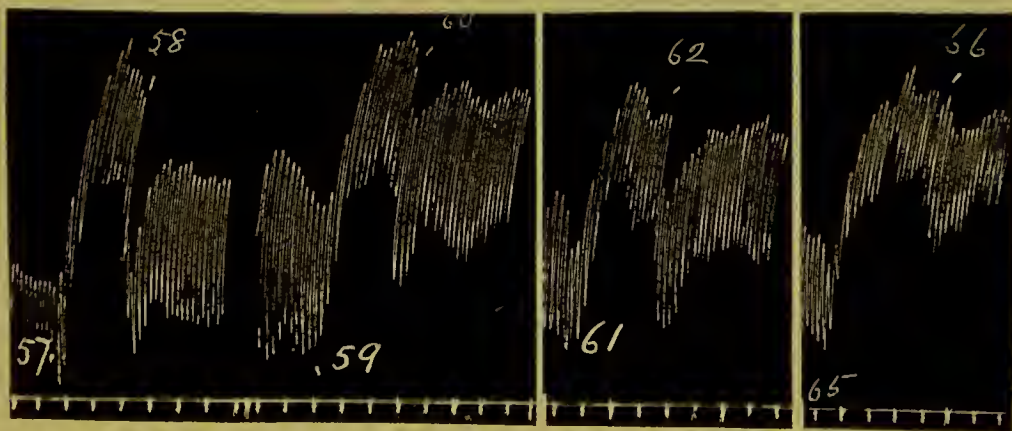


Fig. 15. Intestine tracings. Dog's blood from experiment 5. At 57, 59, 61 and 65 Ringer's solution was replaced by indifferent (arterial) blood, and this at 58, 60, 62 and 66, by the tenth, seventh, eighth and fourth adrenal blood specimens respectively. All the bloods were diluted with three volumes of Ringer's solution.

Experiment 5. Dog (male). Weight, 11.3 kgm. Ether. A cava pocket was formed without tying off the intestinal arteries. A blood pressure tracing was taken from the carotid during collection of the adrenal samples. During collection of some of the samples the vagus was stimulated in the neck.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	BLOOD PRESSURE	REMARKS
	cc.	minutes	seconds	cc.	mm. of Hg.	
1	21.4	1	28	14.6	96	
2	20.4	1	25	12.7	92	Vagus stimulation
3	21.9	2	21	9.3	92	
4	16.6	2	15	7.4	82	Vagus stimulation
5	21.9	2	50	7.7	82	Vagus stimulation*
6	17.7	2	30	7.0	82-70	Vagus stimulation*
7	17.6	3		5.8	65	
8	21.9	3	36	6.0	60	
9	21.7	3	55	5.5	60 falling	
10	22.9	6	15	3.6	falling	
11	12.4	4	33	2.7	28-22	

* The vagus was stimulated during part of the time of collection of these specimens.

Blood was drawn from the jugular vein before collection of the adrenal samples to serve as indifferent blood in the intestine and uterus tests. Combined weight of adrenals, 1.30 gram. Seventy-five eubic centimeters of blood was obtained from the carotid at the end of the experiment. Specific gravity of fifth adrenal specimen, 1.071. Specific gravity of the carotid blood at the end of the experiment, 1.062.

Numerous tracings were taken to compare the effects of the various adrenal blood samples on intestine and uterus segments. A few are reproduced in figures 15 to 17. In figure 15 it is seen that the tenth adrenal specimen (observation 58) inhibits the intestine segment much more powerfully than either the seventh (observation 60) or the eighth (observation 62), and that the seventh and eighth specimens cause a distinctly greater inhibition than the fourth (observation 66). Reference to the protocol will show that the blood flow during collection of the tenth sample was twice as great as during collection of the fourth. When the seventh and eighth specimens were being obtained the rate of blood flow was intermediate in amount. In figure 16, the effects of the fourth (observation 64) and eleventh (observation 68) adrenal blood specimens are compared. A much greater inhibition was produced by the eleventh than by the fourth specimen, which was collected with a blood flow nearly three times as great as during collection of the eleventh sample.

A point of some technical importance may be referred to here. Observations 63 and 64 were made with the same blood sample as observations 65 and 66 (fig. 15), in the same dilution, and very near each other in the series. Yet the curves are not suitable for comparison, since the increase of tone produced at 63 is considerably greater than that at 65. Sometimes a segment rather suddenly changes in its sensitiveness to the tone-increasing action of blood, and we have observed that when the augmentation of tone produced by a given blood or serum is increased, the inhibition caused by a given concentration of epinephrin in blood or serum is also increased. Thus the absolute amount of the drop in the curve at 64 is greater than at 66. This phenomenon, which does not occur very frequently, does not interfere at all with the estimation of epinephrin in blood samples, provided the rule is obeyed that only curves which can be properly compared are used for comparison. After washing the segment more thoroughly with Ringer's solution and allowing a longer interval before application of the blood, it began again to give curves like 65 and 66, which are comparable with the others reproduced in figure 15.

In figure 17, are shown a few of the tracings taken with the same bloods, but on a segment from another rabbit. Again, of course, observations in this series cannot be compared with observations on the same bloods in the series on the other segment. Thus, observations 16 (fig. 17) and 68 (fig. 16) were both obtained with the eleventh adrenal blood specimen and observations 31 (fig. 17), 64 (fig. 16) and 66 (fig. 15) with the fourth specimen. Observations 18 and 23 (fig. 17) were made with the same blood (the ninth adrenal sample) and on the same intestinal segment, but the segment was differently weighted, and they cannot be compared for quantitative purposes.

It is sometimes advantageous after making an epinephrin assay on a segment to change the weight or to change the segment and to repeat the assay. Each curve in the second series will differ from the corresponding curve in the first. If a new segment has been taken, there may be no resemblance between the corresponding curves in the two series, but this will not interfere in the least with the comparison of the

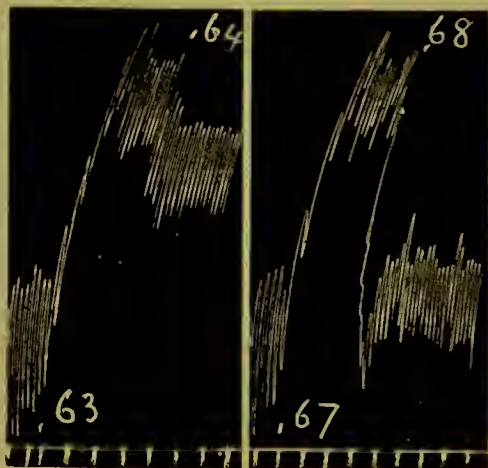


Fig. 16. Intestine tracings. Bloods from the same dog used for figure 15. At 63 and 67 Ringer's solution was replaced by indifferent (arterial) blood and this at 64 and 68 by the fourth and eleventh adrenal blood specimens respectively. The bloods were diluted with three volumes Ringer's solution.

curves in one and the same series, and the relative position of the various blood samples as regards their content of epinephrin will be identical for the two series.

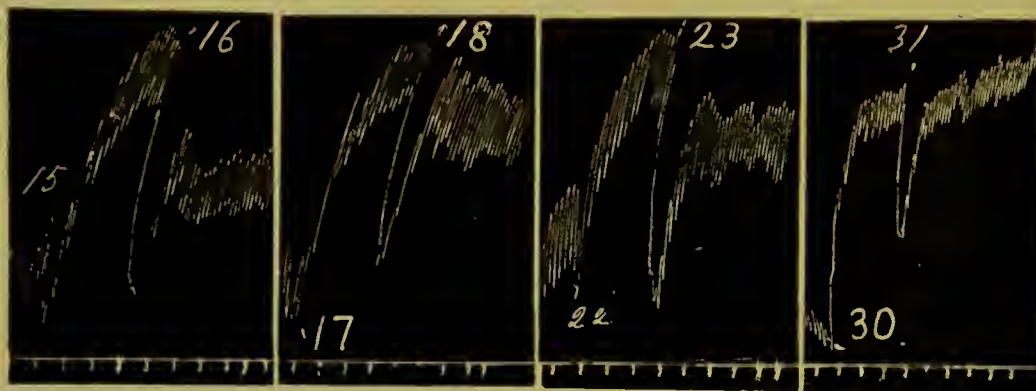


Fig. 17. Intestine tracings. Bloods from the same dog used for figures 15 and 16. The intestine segment was from another rabbit. At 15 and 17 Ringer's solution was replaced by indifferent (jugular) blood, and this at 16 and 18 respectively by the eleventh and ninth adrenal blood specimens. After 18 the weight was increased. At 22 and 30 Ringer's solution was replaced by jugular blood, and this at 23 and 31 by the ninth and fourth adrenal blood specimens, respectively. All the bloods were diluted with three volumes of Ringer's solution.

SUMMARY

It is shown that within the limits of error of the method used for the assay (rabbit intestine and uterus segments) the concentration of epinephrin in the adrenal vein blood collected from a cava pocket varies in different samples from the same animal inversely as the rate of blood flow through the glands during the period of collection, the rate of output of epinephrin per minute being constant under the experimental conditions for a considerable range of blood flow.

The rule fails, of course, when the rate of blood flow is diminished below the value at which the concentration of epinephrin has reached the possible maximum. For this reason determinations of the rate of spontaneous liberation of epinephrin by assays on drawn adrenal vein blood should be made on samples obtained with a rate of flow well above this limiting value.

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86⁷(1264)

Quantitative experiments on the liberation of epinephrin from the adrenals after section of their nerves, with special reference to the question whether epinephrin is indispensable for the organism.

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1. We showed in a previous paper¹ by the blood pressure and eye reactions that after section of the nerve supply of the adrenal no demonstrable liberation of epinephrin was present in cats as long as five weeks after the nerve section.

2. As it is easier to detect very small concentrations of epinephrin by the rabbit intestine and uterus segments, we have made a series of experiments (on 7 cats) in which these tests were used to supplement the eye reactions. In all the animals one adrenal was excised and the nerves of the other cut.

In a cat tested two weeks after the operation, it was shown that the adrenal blood serum could not have contained 1 : 300,000,000, or the blood 1 : 400,000,000, of epinephrin; and that the rate of liberation of epinephrin could not have been at most 0.000001 mgm. per minute for one adrenal. In another cat three weeks after the operation² the serum of the adrenal blood was proved to contain less than 1 : 400,000,000, and the blood less than 1 : 700,000,000 epinephrin. The output of epinephrin per minute could not have been as much as 0.0000009 mgm. per minute, for one adrenal. The segments used for the tests in these experiments were extremely sensitive, and the limits of adrenalin concentrations which could be detected with certainty were care-

¹ *Journal of Pharmacology and Experimental Therapeutics*, 1916, VIII, 479.

² In this animal after the usual operation the left semilunar ganglion and the first ganglion of the lumbar sympathetic chain below the diaphragm were excised.

fully determined. The eye reactions were negative. In these two cats the rate of liberation of epinephrin, if any liberation whatever was going on, must have been several hundred times less than the rate in normal animals under the same experimental conditions.

It is scarcely necessary to point out that experiments yielding completely negative results indicating the absence of epinephrin with very sensitive test objects are much more important for the questions studied than experiments in which small amounts of epinephrin can still be detected. For it is impossible to be certain that when a little epinephrin is found some of the fibers concerned in the liberation may not have escaped section.

3. Since these animals had completely recovered from the operation and behaved in every way like normal animals, it must be concluded that the liberation of epinephrin from the adrenals is not indispensable for life or health, unless indeed the necessary quantity is, even in the adrenal vein blood, below the limits of detection by the methods used. It must be remembered that the epinephrin in the adrenal blood is diluted enormously (probably at least 100 times) in the right heart; so that in these cats the concentration in the arterial blood could not at most have reached 1 : 40 billions and 1 : 70 billions, respectively.

If the liberation of epinephrin is abolished by division in the dorsal cord of the path concerned in it, as our experiments on "Relation of the Spinal Cord to the Spontaneous Liberation of Epinephrin" indicate, this corroborates the conclusion that epinephrin is not indispensable. Numerous animals and men have long survived such lesions.

4. These experiments indicate that the entire liberation of epinephrin from the adrenals is controlled by nerves.

5. In a third cat (8 days after operation) the adrenal vein blood contained epinephrin but in concentration not exceeding 1 : 125,000,000. The output of epinephrin per minute was probably not more at most than one-hundredth of what might be expected in a normal animal.

6. In a cat 15 weeks after the operation it was doubtful if any epinephrin was present in the adrenal vein blood. In two others 15 weeks after operation eye reactions and segment tests showed

the presence of a small amount of epinephrin, the rate of liberation being a mere fraction of the normal. The possibility of regeneration of fibers after this interval must be considered. In the seventh cat (tested two weeks after the operation) the eye reactions were negative. The segment tests revealed a small concentration of epinephrin in the adrenal blood (less than 1 : 30,000,000) corresponding to a rate of liberation of epinephrin per minute of at most one tenth of the normal.

QUANTITATIVE EXPERIMENTS ON THE LIBERATION OF EPINEPHRIN FROM THE ADRENALS AFTER SECTION OF THEIR NERVES, WITH SPECIAL REF- ERENCE TO THE QUESTION OF THE INDISPENSA- BILITY OF EPINEPHRIN FOR THE ORGANISM

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It is known that after section of certain nerves the rate of the spontaneous liberation of epinephrin is greatly reduced. This is very easy to demonstrate in the cat in acute experiments by the method previously described by us (1) (collection of adrenal vein blood in a cava pocket, and the action of the blood when released in eliciting dilatation of the pupil and retraction of the nictitating membrane in the eye previously prepared by Meltzer's procedure, so as to react with great sensitiveness to epinephrin). After section of the fibers coming to the semilunar ganglion, including the splanchnics, or after section of the two sympathetic trunks, including the splanchnics, in the thorax near to the diaphragm, the eye reactions are in general no longer obtained, unless with considerably longer periods of collection of blood than were sufficient to elicit them strongly before the nerve section. The rise of blood pressure associated with the release of blood containing epinephrin is likewise missing after the nerves have been severed. In survival experiments also it was shown that when the right adrenal was excised and the fibers coming to the left semilunar ganglion cut the eye and blood pressure reactions could no longer be detected. Since cats survive this operation indefinitely, and so far as can be seen after recovery from the operation in the same health and vigor as normal animals, the experiments obviously have a bearing

TABLE 2

NUM- BER OF CAT	BODY WEIGHT	WEIGHT OF AD- RENALS	AD- RENAL BLOOD SAMPLE	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
				Grams	In minutes and seconds		Gms. per minute		Per ani- mal per minute	Per kilo per min- ute
					minutes	seconds				
64	kgm. 2.425	grams 0.390	1	0.9		20	2.7	1: 2,000,000 1: 2,000,000 1: 2,000,000	mgm. 0.0006	mgm. 0.00025 0.00025 0.00025
			2	1.9	1	30	1.3			
			3	2.6	2	10	1.3			
			4	4.3	3	20	1.3			
			5	3.3	3		1.1			
			6	4.0	4		1.0			
63	3.41	0.550	1	2.0		55	2.2	1: 2,000,000 1: 2,000,000 1: 2,000,000	0.001	0.0003 0.0003 0.0003
			2	2.9	1	15	2.3			
			3	3.1	1	20	2.4			
			4	3.3	1	45	1.9			
			5	4.0	2	5	2.0			
			6	3.2	2		1.6			
			7	2.0	1		2.0			
			8	6.5	4	25	1.5			
65	2.035	0.338	1	2.5	1		2.5	1: 2,500,000 1: 1,500,000 1: 1,000,000	0.0009	0.00045 0.00045 0.0002
			2	4.5	2		2.2			
			3	3.6	2	45	1.3			
			4	2.5	3	30	0.7			
			5	1.7	4		0.4			
67	2.31	0.440	1	2.0		20	6.0	1: 13,000,000	0.0004	0.0002
			2	10.2	2		5.0			
81	1.98	0.396	1	3.6	1	20	2.8	1: 2,200,000	0.0005	0.00025
			2	9.9	5		2.0			
			3	6.8	6		1.13			
			4	3.0	4	30	0.66			
82	2.66	0.300	1	5.3	2	30	2.1	1: 4,500,000 1: 2,300,000	0.0005	0.0002 0.0002
			2	8.3	4		2.1			
			3	9.0	5		1.8			
			4	8.8	6		1.5			
			5	6.9	7		1.0			
			6	4.5	8		0.56			
83	3.03	0.360	1	4.7	2		2.3	1: 3,200,000 1: 2,000,000	0.0006	0.0002 0.0002
			2	7.8	4		1.9			
			3	10.1	6		1.7			
			4	9.8	8		1.2			
			5	3.5	9		0.4			

TABLE 2—Continued.

NUM- BER OF CAT	BODY WEIGHT	WEIGHT OF AD- RENALS	AD- RENAL BLOOD SAMPLE	BLOOD FLOW			EPINEPHRIN CONCENTRATION	EPINEPHRIN			
				Grams	In minutes and seconds			Gms. per minute	Per ani- mal per minute	per kilo per min- ute	
					minutes	seconds					
84	kgm.	grams	1	5.2	2		2.6	1:4,000,000	0.0006	0.0002	
			2	9.3	4						2.3
			3	11.6	5						2.3
			4	10.6	5						2.1
			5	11.1	6						1.8
			6	5.7	10						0.5
85	3.16	0.422	1	3.6	2		1.8	1:1,700,000	0.0008	0.00025	
			2	5.2	4						1.30
			3	7.3	10						0.73
			4	1.6	8						30
86	2.875	0.680	1	3.8	4		0.95	1:1,500,000	0.0004	0.00015	
			2	4.7	8						0.6
			3	1.6	4						0.4

In all the cats except 84 and 86 all the arteries (renal, coeliac, mesenteric and abdominal aorta) were tied. In 84 and 86 the coeliac and mesenteric arteries were not tied. All the cats except 65 were anesthetized with urethane. Cat 65 was rendered insensitive by increased intracranial pressure. In cat 64, in addition to urethane anesthesia, the intracranial pressure was increased. In cat 67 the unusually low concentration of epinephrin was associated with an exceptionally large blood flow, but no unligated small vein could be found.

nals may be, the concentration of epinephrin in the blood of the adrenal veins (in cats) cannot rise beyond a certain maximum (not very far from 1:1,000,000 as assayed by rabbit segments). When this maximum has once been reached further diminution of the rate of blood flow necessarily leads to a diminished output per minute. When the blood flow is free, and the concentration well below this limiting value, it can be proved that within a wide range the rate of flow and the concentration of epinephrin vary inversely, the rate of liberation of the epinephrin per minute remaining constant. Since when blood is drawn off from a cannula in the cava pocket the rate of flow is nearly always diminished to some extent, as compared with the rate of flow in the eye observations, which do not entail the drawing off of blood, the output as estimated from the epinephrin content in the drawn blood may easily be below the true value unless samples collected with a sufficient blood flow are used for the assay.

The fact that the earlier adrenal specimens usually have a smaller concentration than the later ones, associated with gradual decline in the rate of blood flow, indicates that the cause of the smaller output per minute estimated on intestine and uterus segments in shed adrenal blood, as compared with the output determined by the eye and blood pressure reactions without drawing blood, is not the loss of epinephrin withdrawn from the circulation. For the effect of epinephrin already circulating in the blood, if any appreciable amount at all were present in the general blood, ought to be most marked in the first specimens.

In two experiments (cats 85 and 86, tables 1 and 2) the epinephrin output was estimated both by the eye reactions and in the drawn adrenal blood by the rabbit segments. In cat 85, in which the output, as determined by the eye reactions, was of fair average magnitude, 0.001 mgm. per minute, the adrenal blood assays yielded values not very far inferior, namely, 0.0008 and 0.0007 mgm. in two samples. The blood flows, while the adrenal samples were being collected, were fairly good; although probably below the flow while the eye reactions were being observed. In cat 86, on the other hand, in which the output as determined by the eye reactions, was very good, 0.002 mgm. per minute, the deficiency in the assays of the adrenal blood on the intestine segments was very considerable; and the blood flow during collection of the samples was rather poor—probably much less than during the testing by the eye reactions. It is obvious, then, that if the question whether the output in an animal is below the normal range or not is being considered, it will not do to compare assays made by the one method with assays made by the other; at any rate, unless the differences are considerable. Fortunately for our purpose, the reduction in the epinephrin output by section of the adrenal nerves is so enormous that it makes not the slightest difference whether we compare the residual liberation with normal values obtained by the eye (and blood pressure) reactions or by the rabbit segments.

Before proceeding to the consideration of the experiments, it may be well to point out that as regards the question of a residual secretion of epinephrin by the adrenals after all the secretory

nerve fibers have been cut, as far as is possible, negative results are much more important than positive ones, always provided that the negative results have been obtained on test objects of the highest degree of sensitiveness. For while the course of the bulk of the secretory fibers has been ascertained, and they can be easily severed, it is impossible to be sure in any given operation that some have not escaped.²

Apart from the surgical risks, it is undesirable in acute experiments to go around the glands with an instrument, since not only is the lymph flow thus necessarily interfered with, but the chance of causing liberation of epinephrin by massage is great. Even then it would be impossible to be sure that some of the fibers in question did not pass in along the blood vessels. In survival experiments, although the effect of massage would introduce no error, the glands would be liable to be affected by adhesions as well as by interference with the lymphatics, if the tissues in their immediate neighborhood were freely divided.

Another reason why experiments yielding well determined negative results with very sensitive test objects must for our problem carry greater weight than experiments in which small quantities of epinephrin are still found in the adrenal blood, even after extensive nerve sections, is that the necessary manipulations in obtaining the blood, the exposure of the glands and the possible disturbance of the circulation in them, might cause a small amount of the epinephrin, known to be present in the glands in not less than normal amount, to escape into the blood. This remark is not intended to imply that when due precautions are taken, it is difficult to avoid the liberation of epinephrin in appreciable amounts in the absence of the secretory nerves. On the contrary, we have never had any reason to suspect that when epinephrin was found in any of the adrenal blood samples, except perhaps in the first small sample, it had not been liberated in the normal way. Even in the preliminary sample, always collected apart with the object of allowing any epinephrin already present in the adrenal capillaries or in the cava pocket itself to be washed out, and which not quite accurately we fell

² Elliott, *Journ. Physiol.*, 1912, xliv, 374, has shown that section of the pre-ganglionic fibers of the semilunar ganglion, including the splanchnics, prevents exhaustion of the store of epinephrin under the influence of various conditions. He made no experiments on the question whether after this operation the liberation of epinephrin is suppressed, and assumes, indeed, that "ultimately" it is resumed, since "the decentralized gland suffices to keep the animal alive."

into the habit of speaking of as the "manipulation sample," we generally found no more epinephrin than in succeeding samples; sometimes, indeed, less, on account of a certain amount of ordinary cava blood being retained in the pocket as it was being closed off.

It may be further remarked that as between the acute and the survival experiments greater weight should be attached to the latter, when the absolute amount of any residual epinephrin liberation is in question. The acute experiments were made mainly to get a general idea of the immediate diminution in the rate of liberation when the nerves were severed. It was desired also to see the effect of piece-meal sectioning of the nerve supply. It was recognized, however, that although a marked immediate diminution in the output was to be looked for, the full effect might not be expressed in the epinephrin content of the adrenal vein blood collected during a short experiment. Apart altogether from the possibility that some irritation of the cut nerves might persist, there was the possibility that epinephrin already in the adrenal capillaries might continue to be washed out in small quantities for some time; or even that a certain amount of epinephrin not yet actually in the blood might be in such a situation, or perhaps in such a combination, at the moment of section of the nerves that it could migrate into the blood stream in the next few minutes without the influence of nerves.

Finally, it must not be forgotten that minor differences in the innervation may occur in different individuals, so that a nerve section which in one cat brings the epinephrin output below the threshold of detectability, say by the eye reactions, need not do so in the case of another cat. We have given illustrations of this in a previous paper (2). In this question there is involved, of course, not merely the possibility of slight anatomical variations; but also the known variability in sensitiveness of the test objects, and the degree of activity of the gland at the moment of section of the nerves. If a relatively large liberation were going on at that moment it is conceivable that a small number of fibers remaining uncut might sustain a detectable output, whereas with a gland less responsive to its innervation the same fraction of the total number of fibers might be unable to do so. The possibility must also be taken into account that a small strand might be overlooked in one

operation which was cut in another, a risk, of course, present especially in the survival experiments where aseptic operations had to be performed, although the large number of times the main operation had been previously done greatly reduced this risk. The remarkable agreement in the general result of the survival experiments shows that any variation due to this factor cannot have been great.

ACUTE EXPERIMENTS

Three such experiments were made on cats and two on dogs. The nerve supply of the adrenals, at least the path of the fibers whose section protects the epinephrin store, and of those whose stimulation increases the epinephrin liberation, has been more completely studied in the cat than in the dog. The experiments on cats are therefore more valuable for the determination of the question how far a complete section of the nerves affects the rate of liberation.

Experiment 1. Condensed protocol. Cat. Weight, 2.7 kgm.

10.30 a.m. 3 grams urethane.

12.05 p.m. Collected specimen of jugular blood; tied off cava pocket, also renal, coeliac and mesenteric arteries.

12.55 p.m. Began collection of adrenal samples as follows:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE
	<i>grams</i>	<i>minutes</i>	<i>seconds</i>	<i>grams</i>
1	3.0	2	43	1.1
2	2.5	3	25	0.73
3	2.7	3	25	0.79
4	1.8	7		0.26

Both sympathetics in the thorax were cut at the end of collection of the second blood specimen just at the origin of the splanchnics below the thirteenth rib. The nerves had been isolated on loose ligatures before collection of adrenal blood was begun. Combined weight of adrenals, 0.392 gram.

It is evident from figure 1, that the first adrenal blood specimen (observation 3), collected before section of the sympathetics, contained a much greater concentration of epinephrin than the fourth specimen (observation 7), collected after the section.

The same thing was shown by comparison of the second specimen (collected before the nerve section) with the third specimen, collected immediately after the section. As this experiment was intended to be mainly a preliminary qualitative one, no very exact assay of the epinephrin concentration was made on the intestine segments. However, it was found that even the fourth sample, which on account of the slow flow would normally have been rich in epinephrin, had a concentration much less



FIG. 1. INTESTINE TRACINGS. BLOODS FROM CAT ANESTHETIZED WITH URETHANE

At 2 Ringer was replaced by indifferent (jugular) blood; and this at 3 by the first adrenal blood specimen (collected before section of sympathetics). At 6 Ringer was replaced by jugular blood and this at 7 by the fourth adrenal specimen (collected after section of the sympathetics). All the bloods were diluted with an equal volume of Ringer. As in all the figures, time is marked in half minutes. (Reduced to one-half.)

than 1:24,000,000, and that the rate of liberation of epinephrin per minute after section of the nerves must have been much less than one-thirtieth of its original amount.

Experiment 1 accordingly shows that section of the two sympathetic trunks at the origin of the major splanchnics reduced to a small fraction of its previous amount the output of epinephrin, which, however, was not abolished.

Experiment 2. Condensed protocol. Cat. Weight, 2.64 kgm. Left superior cervical ganglion excised eighteen days before experiment. Condition excellent.

11.00 a.m. 4 grams urethane.

12.45 p.m. Obtained jugular blood. From 1.00 p.m. to 1.25 p.m., completed cava pocket, tying abdominal aorta below renal arteries.

TIME		DURATION OF POCKET		PUPIL DILATATION AFTER	NICITATING MEMBRANE
		minutes	seconds	seconds	seconds
1.30	Pocket experiment.. Nerves coming to both semilunar ganglia cut	1		Very good, 6.4	Very good, 6.4
1.50	Pocket experiment..	1	10	No	No
1.53	Pocket experiment..	2	10	No	Slight, 11
2.04	Both semilunar ganglia excised.....				
2.10	Pocket experiment..	4		Small, 10.2	Small, 10.2
2.20	Lumbar sympathetic cut below diaphragm.....				
2.40	Pocket experiment..	4		Small, 18.6	Small
2.51	Cut sympathetic trunks in thorax at origin of splanchnics.....				
2.55	Pocket experiment..	4	30	No	Slight
3.10	Pocket experiment..	4	30	Very slight 20.4	Very slight, 20.4

3.13 p.m. Began collection of adrenal blood from pocket. Flow very slow. During fifteen minutes only about 0.5 cc. collected. Then clotting occurred and by removing the clots from time to time another 0.5 cc. was obtained.

This experiment shows that after section of the preganglionic fibers coming to the semilunar ganglion on both sides, slight eye reactions for epinephrin were still obtained. But even with a longer time of collection of blood in the cava pocket the reactions were much reduced. That is to say, the rate of liberation of epinephrin was markedly diminished. After excision of both semilunar ganglia and consequent division of any strands

which might have escaped section before, the time of collection had to be still further increased to give a noticeable reaction. Section of the lumbar sympathetic chain, one ganglion below the diaphragm, caused no further alteration in the reaction. When both sympathetics were now divided in the thorax, a very slight eye reaction was still elicited with a long period of collection.

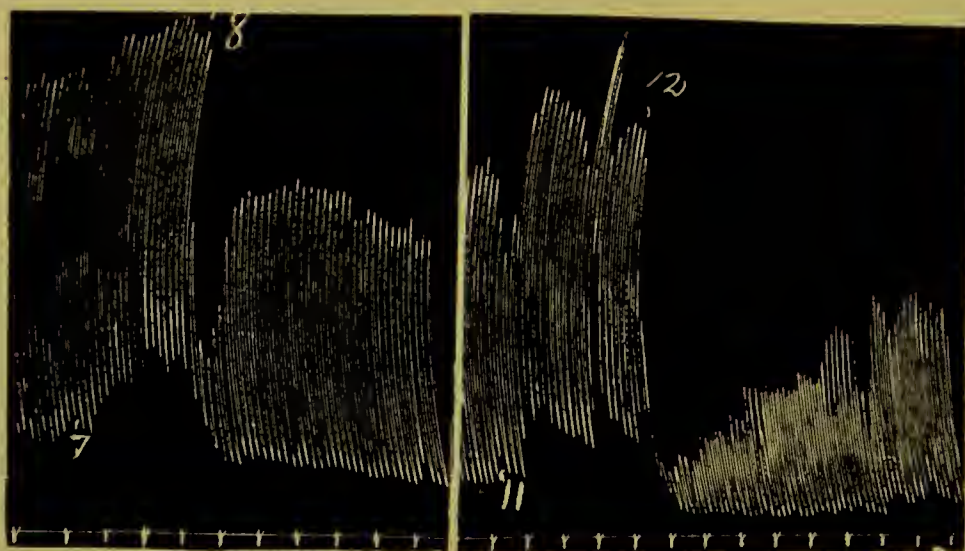


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT ANESTHETIZED WITH URETHANE

At 7 Ringer was replaced by jugular blood, and this at 8 by an adrenal blood specimen collected with very slow blood flow after section of nerves to both semi-lunar ganglia and section of sympathetics in thorax. At 11 Ringer was replaced by jugular blood, and this at 12 by blood containing 1:1,600,000 added adrenalin. All the bloods were diluted with three volumes Ringer before application to the segments. (Reduced to two-thirds.)

A sample of blood obtained from the cava pocket after section of all these nerves contained much less than 1:1,660,000 of epinephrin (fig. 2). The adrenalin assay on intestine segments showed further that the concentration in this blood was less than 1:3,300,000, but more than 1:5,000,000. The flow was very slow when the blood was being collected, doubtless much slower than when the eye observations were being made. In the cat with intact nerves, a considerably greater concentration would be expected with such a small flow. Even if none of the

epinephrin in the blood was due to "manipulation," the rate of liberation must have been reduced by the nerve section, probably to one-fiftieth of its normal amount, as estimated on intestine segments. The rate of liberation per minute does not vary much in the course of an experiment, even when the blood flow diminishes, so long as the concentration is well below the possible maximum, as in this case, increased concentration compensating for lessened flow.

Experiment 3. Condensed protocol. Cat. Weight, 1.41 kgm. Left superior cervical ganglion excised three weeks before the experiment. Condition excellent.

11.20 a.m. 3 grams urethane.

12.45 p.m. Inserted tracheal cannula and made cava pocket, tying off renal arteries and abdominal aorta below renals. The left nictitating was retracted, and nictitating reactions could not be used.

TIME		DURATION OF POCKET		PUPIL DILATATION AFTER
		minutes	seconds	seconds
1.20	Pocket experiment.....	1		Good 6.8
1.22	Pocket experiment with left adrenal vein clipped.....	2		Good 7.4
1.30	Right sympathetic in thorax cut at origin of splanchnic			
1.37	Pocket experiment; left adrenal vein clipped.....	2		No
1.42	Left sympathetic cut in thorax one ganglion above splanchnic origin.			
1.43	Pocket experiment.....	2		No
1.46	Pocket experiment.....	3	10	No

Inserted cannula into right iliac vein, and collected adrenal blood: First specimen, 1 gram in 16.5 minutes (0.06 gram per minute); second specimen, 1.4 grams in 35 minutes (0.04 gram per minute). The blood flow was very slow. While these specimens were being collected, a specimen of jugular blood was obtained. After collection of the adrenal blood a specimen of arterial blood was got from the abdominal aorta, with the cava pocket still closed. Combined weight of adrenals, 0.31 gram.

In this experiment, the eye reaction could no longer be elicited after section of both sympathetic trunks, including the major and minor splanchnics in the thorax. Adrenal vein blood collected after the nerves had been severed showed a concentration of epinephrin by rabbit intestine segment tests of not more than 1:1,500,000. As the flow was extremely slow (0.05 gram per minute, or one-twentieth of an ordinary flow), the relatively high rate of concentration is what would be expected so long as any appreciable amount of epinephrin was being given off. The rate of liberation, however, after the nerve section was only one-tenth to one-twentieth of the normal rate (about 0.00003 mgm. per minute, or 0.00002 mgm. per kilo per minute) (see table 2). The epinephrin assay in this experiment was not entirely satisfactory, because there was reason to believe from the uterus tests that a part of the inhibition of the intestine segment was due to some other substance than epinephrin. It is known that sometimes in asphyxia such a substance appears in venous blood, and even in arterial blood; and its appearance in this experiment might have been associated with the very small blood flow at the time of collection of the adrenal specimens. If this was the case the reduction in the rate of epinephrin output must have been even greater than the calculated reduction. On the other hand, since as already pointed out there is a limit of epinephrin concentration not very far above that found in the adrenal blood in this case, and which is never exceeded in our experience no matter how small the rate of blood flow may be, the question may fairly be asked whether with extremely small flows it is legitimate to assume that the gland is liberating as much epinephrin in response to the residual innervation as it would have done had the flow been larger. As regards our main conclusion, however, this objection has no weight. For not only were the pupil reactions abolished in this experiment by the nerve sections at a time when the blood flow was good, although quite strongly elicited before the nerves were cut; but what is more important exceedingly small blood flows were found associated in some of the survival experiments either with remarkably low concentrations of epinephrin, as determined by the segment

tests, or with a complete absence of epinephrin in detectable amount.

Although there was no reason to assume that the small residual output of epinephrin in these acute experiments was due to anything else than some small uncut remnant of nerve fibers, two acute experiments were made on dogs in order to take advantage of the larger blood flow through the adrenals, which might be expected to wash out more thoroughly any epinephrin already liberated before the various nerve sections. As already stated, however, this advantage was counterbalanced by the less precise knowledge of the nerve paths in the dog; and although the nerve sections practiced reduced the rate of liberation of epinephrin to a mere fraction of its initial amount, a substantial residual output remained.

Experiment 4. Condensed protocol. Dog. Weight, 7.75 kgm. Anesthetized with ether.

9.10 a.m. Tracheal cannula inserted. Specimen of jugular blood obtained. Right adrenal gland excised. Cava pocket made with a cannula in each iliac vein. The following specimens of adrenal blood were obtained through the right iliac cannula: First adrenal specimen, 5 grams in 1 minute, 30 seconds (3.4 grams per minute); second specimen, 7.2 grams in 2 minutes, 16 seconds (3.2 grams per minute).

10.15 a.m. Cut left major and minor splanchnics in abdomen; dissected around left adrenal, cutting nerve connections; excised three lumbar sympathetic ganglia, including chain from diaphragm downward. Then collected through left iliac cannula the following adrenal blood specimens: Third adrenal specimen, 4.3 grams in 5 minutes, 10 seconds (0.8 gram per minute); fourth specimen, 3.0 grams in 6 minutes, 30 seconds (0.5 gram per minute). Pulled out clot from cannula and flow improved. Collected the following specimens: Fifth adrenal specimen, 5.0 grams in 4 minutes, 10 seconds (1.2 grams per minute); sixth specimen (6.5 grams in 6 minutes (1.1 grams per minute); seventh specimen, 4.5 grams in 4 minutes (1.1 grams per minute); eighth

specimen, 3.5 grams in 4 minutes, 30 seconds (0.9 gram per minute). During the latter part of the collection of the eighth specimen, the left sympathetic was cut in the thorax at origin of the splanchnics. The following adrenal blood specimens were then collected: Ninth specimen, 4.8 grams in 4 minutes, 30 seconds (1.1 grams per minute); tenth specimen, 3.8 grams in 8 minutes (0.5 gram per minute). Arterial blood was now obtained from the abdominal aorta, the cava pocket being still closed off.

The rabbit segment tests showed that the concentration of epinephrin in the adrenal blood collected before the nerve

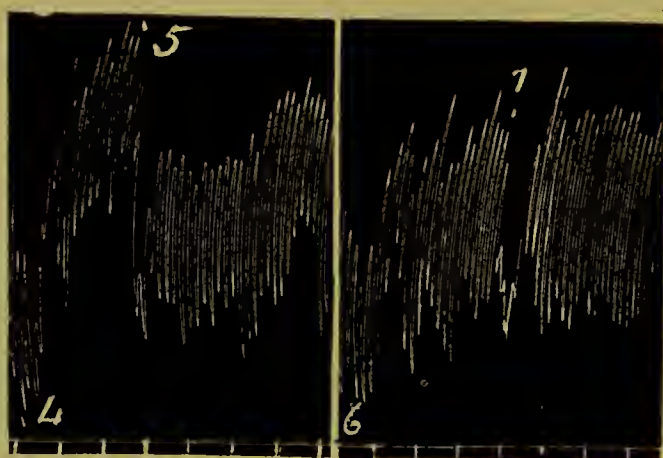


FIG. 3. INTESTINE TRACINGS. BLOODS FROM DOG ANESTHETIZED WITH ETHER, AND WITH RIGHT ADRENAL EXCISED

At 4 Ringer was replaced by indifferent (jugular) blood, and this at 5 by the second adrenal specimen (collected before section of nerves). At 6 Ringer was replaced by jugular blood, and this at 7 by the sixth adrenal specimen (collected after denervation of the left adrenal). The bloods were diluted with an equal volume of Ringer. (Reduced to two-thirds.)

sections was decidedly greater than in the blood collected after the nerve sections (figs. 3 to 5). Some of the tracings taken for assaying the amount of epinephrin in adrenal samples before and after division of the nerves are reproduced in figures 4 and 5. The concentration in the second sample (fig. 3, observation 5) is less than 1:5,000,000, much greater than 1:20,000,000, and not far from 1:7,000,000 (fig. 4, observations 9, 11, 15). A

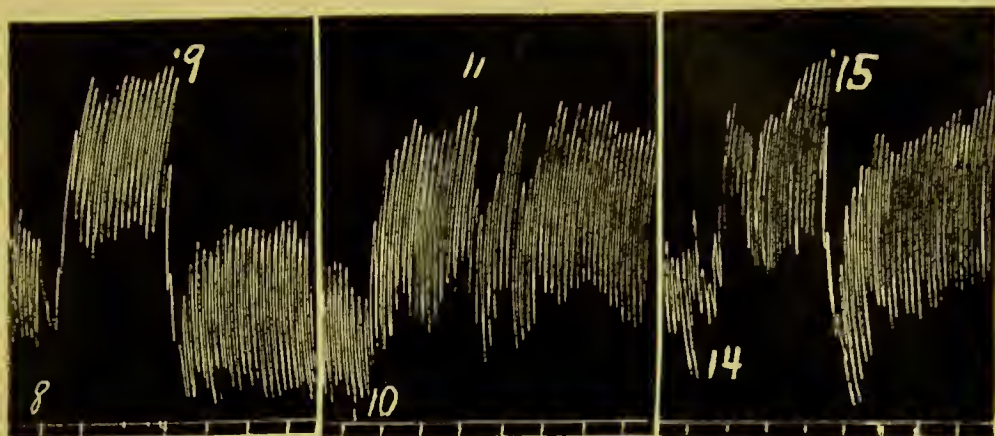


FIG. 4. INTESTINE TRACINGS. BLOODS FROM SAME DOG AS IN FIGURE 3

At 8 Ringer was replaced by jugular blood diluted with an equal volume of Ringer, and this at 9 by jugular blood made up to 1:5,000,000 adrenalin, and then diluted with an equal volume of Ringer. At 10 and 14 Ringer was replaced by jugular blood diluted with an equal volume of Ringer and this at 11 and 15, respectively, by jugular blood made up to 1:20,000,000 adrenalin, and to 1:7,000,000 adrenalin, and then diluted with an equal volume of Ringer. (Reduced to two-thirds.)

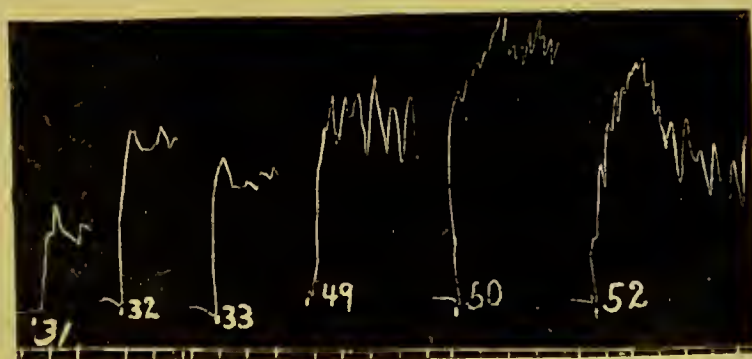


FIG. 5. UTERUS TRACINGS. BLOODS FROM SAME DOG AS USED IN FIGURES 3 AND 4

At 31 Ringer was replaced by indifferent (arterial) blood, at 32 by the second adrenal specimen (collected before section of nerves); at 33 by the sixth adrenal specimen (collected after section of nerves). These bloods were undiluted. At 49 Ringer was replaced by the tenth adrenal specimen (collected after section of the nerves). The blood was diluted with an equal volume of Ringer. At 50 Ringer was replaced by jugular blood to which was added adrenalin to make up 1:10,000,000, and the blood then diluted with an equal volume of Ringer. At 52 Ringer was replaced by jugular blood to which was added adrenalin to make up 1:20,000,000, the blood being then diluted with an equal volume of Ringer. (Reduced to one-half.)

test made with the second adrenal sample immediately after observation 15 gave a curve more nearly resembling that of observation 15, but showing slightly smaller inhibition of the intestine. The sixth adrenal sample contained obviously much less than 1:7,000,000 epinephrin. Another observation on the intestine, not reproduced, showed that the concentration in this sample was greatly inferior to 1:10,000,000. Observation 11 (fig. 4) indicates that the concentration in the sixth sample was not far from 1:20,000,000. This was confirmed on the uterus. Observation 49 (fig. 5), indicates that the effect of the tenth adrenal sample was much less than 1:10,000,000 adrenalin (observation 50), and not very different from 1:20,000,000 (observation 52). On the intestine it was shown, on tracings not reproduced, that the tenth sample caused a slightly greater inhibition than the sixth sample, and this was corroborated by uterus tracings. The rate of flow when the second sample was being collected was about three times as great as during collection of the sixth sample. The concentration of the sixth sample being approximately one-third that of the second, the rate of liberation of epinephrin per minute must have been reduced at least to one-ninth of its original value in consequence of the nerve sections. It must be remembered that the minute-output of epinephrin is that due to one adrenal only. The concentration, however, would not be affected by this circumstance, since one adrenal having been excised, the blood flowing into the cava pocket was of course correspondingly reduced.

It may appear somewhat surprising that in spite of the drastic procedure adopted to sever the secretory nerves of the left adrenal, the rate of liberation of epinephrin was still a substantial fraction of its initial value. Subsequent section of the sympathetic trunk in the thorax, as was to be expected, did not alter this fraction perceptibly. It is quite possible, as suggested before, that the attempt to cut fibers close to the adrenal caused such disturbance in the gland by altering the circulation, and perhaps in other ways, that some epinephrin continued to be discharged during the remainder of the experiment independently of the central innervation.

In the next experiment disturbance of the adrenal was avoided by dividing the sympathetics in the thorax just below the twelfth rib. This operation at once reduced the rate of output of epinephrin to not more at most than one-twelfth of its initial value. That the residual liberation was sustained in the same way as the normal initial output, that is, through nerves which had escaped section, is suggested by the fact that after section of the nerves successive specimens of adrenal vein blood (and serum) showed the same progressive increase in the concentration of epinephrin as before the section. The reduced rate of liberation per minute therefore remained practically independent of the rate of blood flow, just as the original rate did before the nerve section, the concentration varying approximately in the inverse ratio of the blood flow.

Experiment 5. Condensed protocol. Dog. Weight, 18.35 kgm. Anesthetized with ether.

10.40 a.m. Tracheal and jugular cannulae inserted.

10.50 a.m. Obtained jugular blood. Cava pocket made, and cannula inserted in the right iliac vein. The following specimens of adrenal blood were collected: First specimen, 36.2 grams (time record lost); second specimen, 30 grams in 1 minute, 10 seconds (26.1 grams per minute); third specimen, 26.0 grams, in 1 minute, 20 seconds (19.5 grams per minute). Cut both sympathetics in thorax one ganglion above origin of splanchnic. Collected the following specimens of adrenal blood: Fourth specimen and fifth specimen not satisfactorily collected; sixth specimen, 22 grams in 1 minute, 37 seconds (13.6 grams per minute); seventh specimen, 33 grams in 2 minutes, 25 seconds (13.2 grams per minute). Combined weight of adrenals, 1.480 grams. The bloods were centrifuged and the sera tested on rabbit segments.

Of the numerous observations made to assay the epinephrin content of the sera only four are reproduced (figs. 6 and 7). Figure 6 shows that the third adrenal sample taken just before section of the nerves had a much greater concentration of epinephrin than the sixth sample, collected after the nerve section.

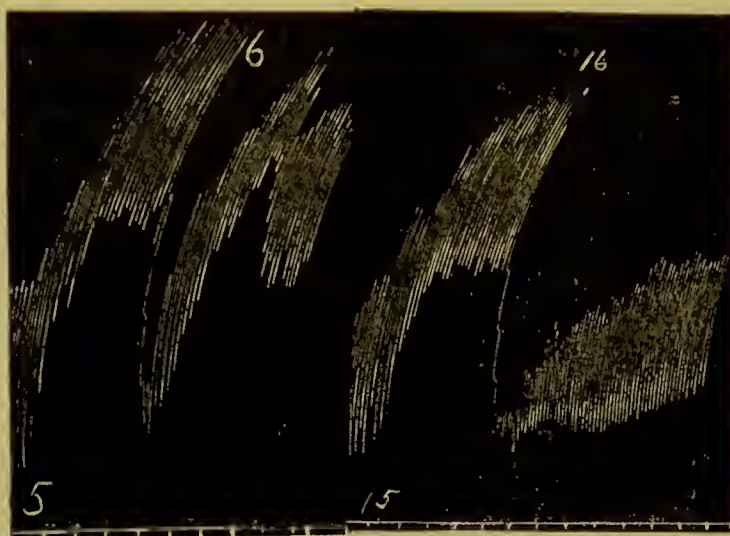


FIG. 6. BLOODS FROM DOG ANESTHETIZED WITH ETHER

At 5, Ringer was replaced by the serum of jugular blood, and this at 6 by the serum of the sixth adrenal blood specimen (collected after section of the sympathetics in the thorax). At 15 Ringer was replaced by the serum of jugular blood, and this at 16 by the serum of the third adrenal specimen (collected before section of the sympathetics). All the sera were diluted with an equal volume of Ringer. (Reduced to one-half.)

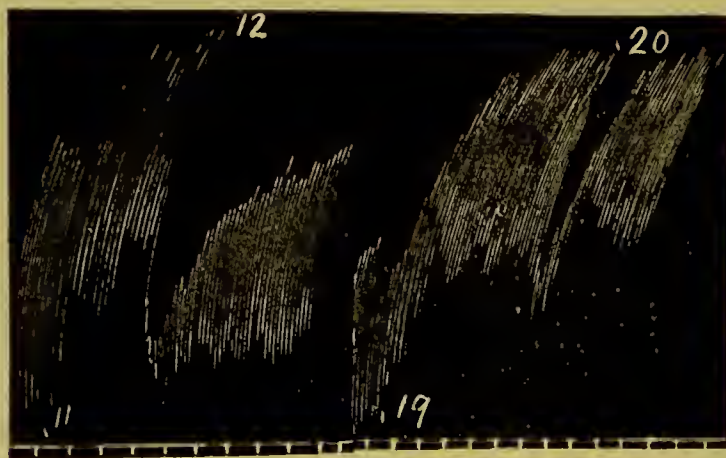


FIG. 7. INTESTINE TRACINGS. BLOODS FROM SAME DOG AS USED IN FIGURE 6

At 11 Ringer was replaced by serum of jugular blood, and this at 12 by serum of jugular blood to which had been added adrenalin to make up 1:3,000,000. At 19 Ringer was replaced by serum of jugular blood, and this at 20 by serum of jugular blood to which had been added adrenalin to make up 1:20,000,000. All the sera were diluted with an equal volume of Ringer. The concentrations of adrenalin given are the concentrations in the serum before this dilution. (Reduced to one-half.)

Observation 12 (fig. 7) indicates that the concentration in the serum of the third sample was decidedly greater than 1:3,000,000. The concentration in the serum of the sixth sample was somewhat greater than 1:20,000,000 (observation 20). Other observations with various concentrations of adrenalin confirmed the conclusion that the concentration in the sixth sample was not far from one-eighth of that in the third sample. The rate of flow of the third sample was approximately in the ratio of 3:2 to that of the sixth. Therefore, the rate of liberation of epinephrin per minute was diminished to about one-twelfth by the nerve section.

The results of the acute experiments can be summed up in a sentence. In none of them have we failed to find in the adrenal vein blood after section of the nerves a sufficient concentration of epinephrin to be detected by the rabbit intestine and uterus segments even where the eye reactions were negative. In all the experiments the rate of liberation was reduced by the nerve sections to a small fraction of the normal output.

SURVIVAL EXPERIMENTS

Seven of these are recorded here, all on cats. In all both eye reactions and segments tests were employed. One of the animals was tested 8 days after the nerve section (experiment 6); two after 16 and 15 days, respectively (experiments 7 and 8); one after 3 weeks (experiment 9); and three after 15 weeks (experiments 10, 11 and 12). In all the animals one superior cervical ganglion was removed about a week before the tests.

Experiment 6. Condensed protocol. Cat. Weight, 2.0 kgm. Right adrenal gland excised, nerve connections to left semilunar ganglion severed, and left superior cervical ganglion excised, 8 days before the experiment. Condition good.

- 9.30 a.m. 3 grams urethane.
- 10.30 a.m. Tracheal cannula inserted, cava pocket made with cannula in lower end.
- 11.10 a.m. Sample of jugular blood obtained.
- 11.25 a.m. Pocket experiment, 1 minute, 30 seconds, no pupil or nictitating reaction.

- 11.28 a.m. Pocket experiment, 2 minutes, no pupil or nictitating reaction.
- 11.32 a.m. Pocket experiment, 3 minutes, questionable eye reactions.
- 11.37 a.m. Pocket experiment, 4 minutes, pupil and nictitating reactions very faint, if any.
- 11.45 a.m. Collected adrenal blood specimens as follows: First specimen, 1.7 grams in 3 minutes (0.57 gram per minute); second specimen, 3.4 grams in 7 minutes, 45 seconds (0.44 gram per minute); third specimen, 4.9 grams in 17 minutes (0.29 gram per minute); fourth specimen, 1.9 grams in 11 minutes, 30 seconds (0.16 gram per minute). Blood obtained from abdominal aorta with cava pocket still clipped off. Right adrenal weighed 0.165 gram, and contained 0.22 mgm. epinephrin. Left adrenal weighed 0.178 gram, and contained 0.27 mgm. epinephrin.

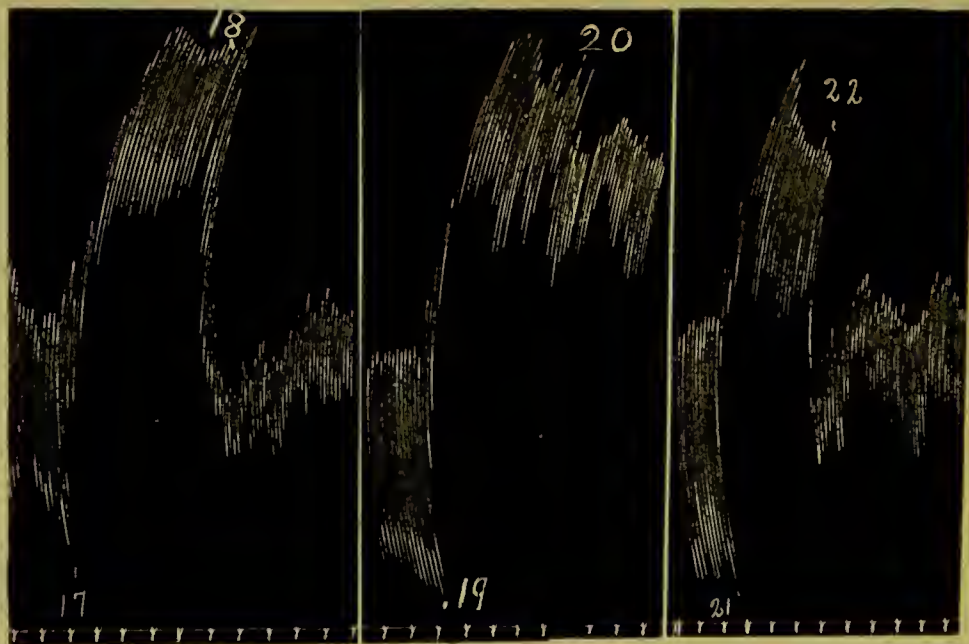


FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL EXCISED AND NERVES OF LEFT SEVERED

Anesthetized with urethane. At 17 Ringer was replaced by indifferent (arterial) blood, and this at 18 by the first adrenal specimen. At 19 Ringer was replaced by arterial blood and this at 20 by the second adrenal specimen. At 21 Ringer was replaced by arterial blood, and this at 22 by the third adrenal specimen. All bloods were diluted with an equal volume of Ringer before being applied to the segment. (Reduced to three-fifths.)

The eye reactions were practically negative. The segment tests proved that the concentration of epinephrin in the adrenal blood was very small even in the samples with the slowest blood flow. A few of the numerous tracings taken are reproduced in figures 8 and 9. Comparison of observations 20 and 28 indicates that the concentration in the second adrenal sample was not more

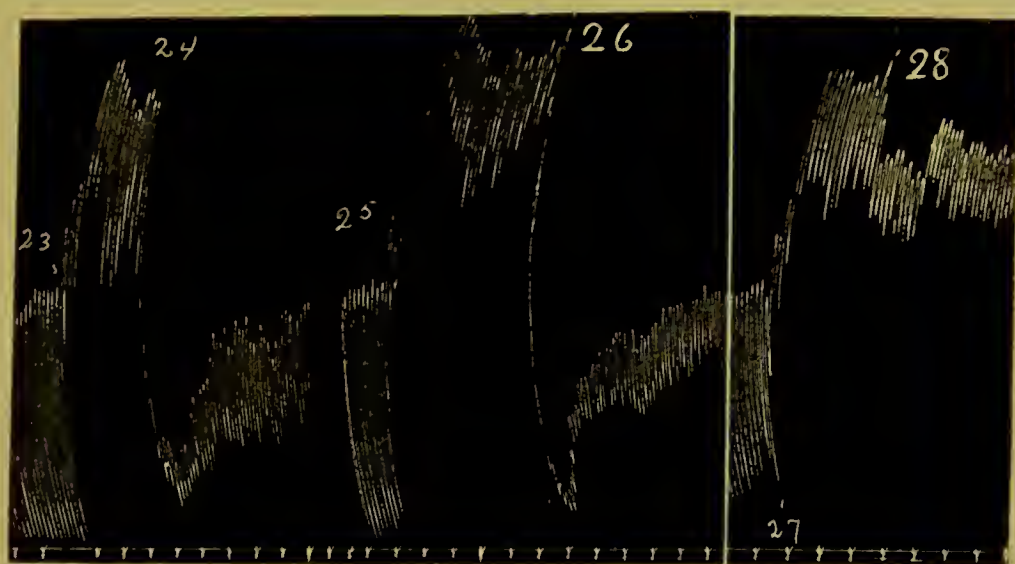


FIG. 9. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURE 8

At 23 Ringer was replaced by arterial blood, and this at 24 by arterial blood containing 1:20,000,000 adrenalin. At 25 Ringer was replaced by arterial blood and this at 26 by arterial blood containing 1:50,000,000 adrenalin. At 27 Ringer was replaced by arterial blood, and this at 28 by arterial blood containing 1:125,000,000 adrenalin. The indifferent (arterial) bloods were diluted with an equal volume of Ringer and after addition of the adrenalin to the arterial blood to make up the concentrations named, the adrenalin blood was also diluted with an equal volume of Ringer before application to the segments. (Reduced to three-fifths.)

than 1:125,000,000. It was very much less than 1:50,000,000 (observation 26). The intestine segment was quite sensitive to adrenalin. Indifferent blood made up with adrenalin to 1:50,000,000, the mixture being then diluted with its own volume of Ringer before application to the segment, produced an effect probably not far from maximal as regards the initial diminution of tone. The greater effect of a 1:20,000,000 concentration

similarly diluted with Ringer (observation 24) is displayed simply in the greater persistency of the diminished tone. The third sample (observation 22) had a concentration of less than 1:50,000,000, probably no more than 1:75,000,000. The rates of flow of the second and third samples were in the ratio of 3:2, or approximately in the inverse ratio of the concentrations. It follows that the rate of liberation of epinephrin was proceeding uniformly during the collection of these samples. The greater concentration in the first adrenal sample may be assumed to be due to a small amount of epinephrin, the liberation of which was associated with the necessary manipulation in closing off the pocket. This would tell especially when the genuine output of epinephrin was very small, as in this experiment.

The uterus tests confirmed the conclusion that the inhibition of the intestine was due altogether to epinephrin.

Taking the rate of flow of the second sample as 0.43 cc. per minute, and the concentration of epinephrin in it as 1:125,000,000, we get 0.0000035 mgm. per minute as the output of epinephrin from the one adrenal, i.e., 0.000007 mgm. for the two adrenals (0.000003 mgm. per kilo of animal per minute). This is at most no more than one-hundredth of the normal output as determined by rabbit segments in adrenal blood (table 2), and not more than one-two-hundredth of the normal output as determined by the eye reactions (table 1).

Experiment 7. Condensed protocol. Cat. Weight, 2.75 kgm. The right adrenal gland was excised and the nerve connections to the left semilunar ganglion severed 2 weeks before the experiment. The left superior cervical ganglion was excised 9 days before the experiment. Condition excellent.

11.00 a.m. 4 grams urethane.

12.30 p.m. Tracheal cannula inserted. Jugular blood obtained.
Cava pocket made.

1.22 p.m. Pocket experiment, 2 minutes. No pupil or nictitating reactions.

1.26 p.m. Pocket experiment, 3 minutes, no pupil or nictitating reactions.

1.30 p.m. Collected the following adrenal blood specimens: First specimen, 2.7 grams in 1 minute, 30 seconds (1.8 grams per minute); second specimen, 5.7 grams in 3 minutes, 30 seconds (1.6 grams per minute); third specimen 7.4 grams in 7 minutes (1 gram per minute). Blood obtained from abdominal aorta. Specific gravity of this blood, 1028. The blood pressure during collection of the adrenal specimens was good. Right adrenal weighed, 0.110 gram, and contained 0.08+ mgm. epinephrin. Left adrenal weighed, 0.214 gram, and contained 0.18 mgm. epinephrin.



FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL EXCISED AND NERVE CONNECTIONS OF LEFT CUT

Anesthetized with urethane. At 5 and 15 Ringer was replaced by indifferent (arterial) blood, and this at 6 and 16 by the second and third adrenal specimens, respectively. The bloods were all diluted with an equal volume of Ringer before application to the segments. (Reduced to one-half.)

The eye reactions in experiment 7 were negative. Some specimens of tracings from the segments tests are reproduced in figures 10 and 11. From these and other observations on the intestine segments, it was determined that the second adrenal blood specimen (observation 6) was weaker than 1:30,000,000 adrenalin (observation 12), much weaker than 1:15,000,000

(observation 10). The third adrenal specimen (observation 16) had a greater concentration than the second, corresponding to the lessened blood flow, but it was still not as strong as 1:30,000,000. The amount of epinephrin liberated per minute was therefore not as much as 0.00003 mgm. for the one adrenal, or 0.00006 mgm. for both glands (0.00002 mgm. per kilo of animal per

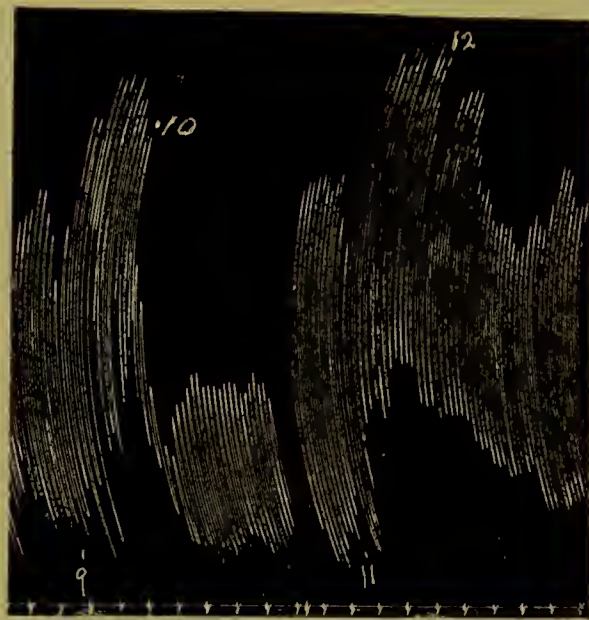


FIG. 11. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURE 10

At 9 and 11 Ringer was replaced by arterial blood, and this at 10 and 12 by arterial blood to which had been added adrenalin to make up 1:15,000,000 and 1:30,000,000, respectively. All the bloods were diluted with an equal volume of Ringer before application to the segment. The concentrations of adrenalin given are the concentrations in the blood before this dilution. (Reduced to one-half.)

minute), i.e., not one-tenth of the normal discharge, as determined in drawn adrenal blood on rabbit segments, and not more than one-twentieth to one-thirtieth of the amount as determined by eye reactions.

The next two experiments of the survival series (experiments 8 and 9) yielded absolutely decisive results.

Experiment 8. Condensed protocol. Cat. Weight, 2.155 kgm. The right adrenal was excised and the nerve connections to the left

semilunar ganglion severed two weeks before the experiment. The left superior cervical ganglion was excised one week before the experiment. Condition good.

11.00 a.m. 4 grams urethane.

12.30 p.m. Tracheal cannula inserted. Cava pocket made.

1.05 p.m. Pocket experiment, 45 seconds. No eye reactions.

1.07 p.m. Pocket experiment, 1 minute, 50 seconds. No eye reactions.

1.10 p.m. Pocket experiment, 3 minutes 30 seconds. No eye reactions.

1.20 p.m. Injected into jugular 0.5 cc. of 1:20,000,000 adrenalin.
Very good pupil and nictitating reactions.

1.21 p.m. Injected into jugular, 1.0 cc. of 1:20,000,000 adrenalin.
Excellent pupil and nictitating reactions.

1.25 a.m. Injected into jugular less than 0.5 cc.³ of 1:20,000,000 adrenalin. Good pupil and nictitating reactions.

1.30 p.m. Inserted cannula in lower end of cava pocket, and collected the following adrenal blood specimens: First specimen, 2.0 grams in 1 minute, 15 seconds (1.6 grams per minute); second specimen, 1.9 grams in 1 minute, 45 seconds (1.1 grams per minute); third specimen, 3.7 grams, in 5 minutes, 10 seconds (0.7 gram per minute); fourth specimen, 2.8 grams in 7 minutes (0.4 gram per minute). Blood obtained from jugular vein and also from abdominal aorta, while the cava pocket was still shut off from the circulation.

2.00 p.m. Pocket experiment, 2 minutes. No eye reactions.

2.03 p.m. Pocket experiment, 3 minutes, 30 seconds. No eye reactions.

2.08 p.m. Pocket experiment, 5 minutes. No eye reactions.

2.20 p.m. Injected into jugular 0.5 cc., of 1:40,000,000 adrenalin.
Slight pupil reaction in 20.4 seconds.

2.23 p.m. Injected into jugular 0.5 cc., of 1:40,000,000 adrenalin.
Slight pupil reaction in 30 seconds. Capacity of cava pocket slackly filled, 0.64 gram. The arterial blood contained 72 per cent of serum (by hemaetocrit). Right adrenal weighed, 0.150 gram, and contained 0.25 mgm. epinephrin (assayed when excised). Left adrenal weighed, 0.230 gram, and contained 0.24 mgm. epinephrin (assayed after the experiment).

³ It was intended to inject 0.5 cc., but it did not wash in completely with the Ringer's solution (about 1 cc.) which followed the injection.

The eye reactions in experiment 8 were negative, although they could be elicited by injection of 0.00001 mgm. of adrenalin into the jugular vein, and were strongly obtained with less than 0.000025 mgm. Even with collection in the pocket for 5 minutes, no eye reactions were got, i.e., not even 0.000002 mgm. epinephrin per minute was being liberated by the one adrenal (i.e. 0.000002 mgm. per kilogram of body weight for the two adrenals). This is not more than one-three-hundredth of the normal output, as estimated by eye reactions.

A few specimens of the tracings from the rabbit intestine and uterus segment tests are given in figures 12 to 15. They showed that if any epinephrin whatever was present in the adrenal vein blood, which was not certain, it could only have existed in a concentration already almost beyond the limit of detectability by the extremely sensitive intestine and uterus segments worked with. For example, if the slight dip at observation 21 (fig. 13) is due to epinephrin, and not merely to the change of liquid around the preparation, it indicates that the concentration of epinephrin in the third adrenal blood specimen could not have been as much as 1:100,000,000 (observation 19). That with this segment a slight dip in the curve could be caused by the mere change of the blood surrounding the segment without any epinephrin being present, is illustrated in observation 9 (fig. 12), where arterial blood from one cat replaced arterial blood from another. Observation 17 (fig. 12) on the third adrenal sample reveals no definite inhibition. In observation 11, where the first adrenal blood replaced indifferent (arterial) blood, there is only a slight dip and a delayed increase of tone, notwithstanding the possibility that a little epinephrin might have been liberated into this sample by manipulation. Even the serum of the adrenal blood, which, as has been previously shown (3), is richer in epinephrin than the blood (containing, indeed, practically the whole of it), caused no definite inhibition of the intestine segments (fig. 14, observation 26), while 1:60,000,000 adrenalin in indifferent blood produced good inhibition (observation 29), and the effect of 1:100,000,000 (observation 31) was distinct.

On uterus segments, it was shown that adrenalin in the con-

centration of 1:200,000,000 (fig. 15, observations 41 and 45) caused a much greater increase of tone than the undiluted serum of the fourth adrenal specimen, notwithstanding the fact that the adrenalin was made up in an indifferent blood diluted with

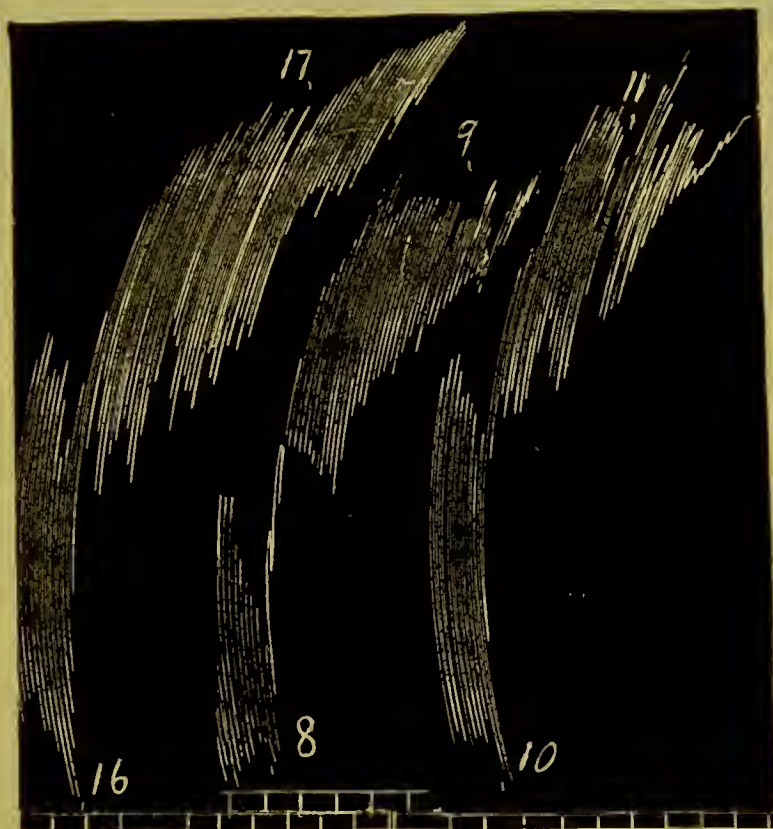


FIG. 12. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL EXCISED AND NERVES OF LEFT CUT

Anesthetized with urethane. At 8 Ringer was replaced by arterial blood, and this at 9 by the same arterial blood. At 10 Ringer was replaced by arterial blood, and this at 11 by the first adrenal blood specimen. At 16 Ringer was replaced by arterial blood and this at 17 by the third adrenal specimen. All the bloods were diluted before application to the segment with two volumes Ringer; except at observations 16 and 17, where the bloods were diluted with one volume Ringer. (Reduced to two-thirds.)

its own volume of Ringer, and that the ordinary serum effect on the uterus would therefore be less than the serum effect in observation 43. The indifferent blood in this dilution (tracings not reproduced), and also the adrenal vein blood of the second speci-

men (observation 38) similarly diluted, caused a much smaller effect on the uterus segment than the undiluted serum of the fourth adrenal specimen, and also a smaller effect than the indifferent blood (diluted with one volume Ringer) to which adrenalin in the concentration of 1:300,000,000 (observation 44) had been added. It is probable that even 1:400,000,000 (observation 42) could be detected by this segment.

There is no doubt, then, that the serum of the fourth adrenal specimen contained much less than 1:200,000,000, and even less than 1:300,000,000. The proportion of serum in the blood

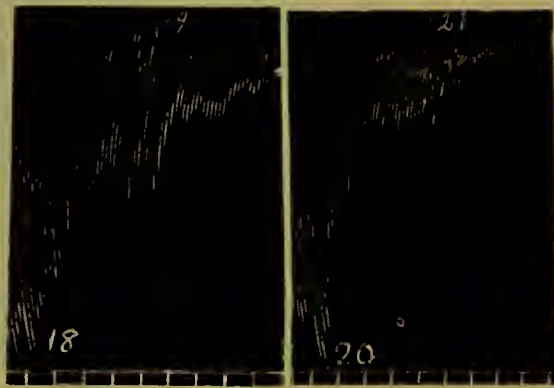


FIG. 13. INTESTINE TRACINGS. BLOODS FROM THE SAME CAT USED FOR FIGURE 12, BUT WITH SMALLER MAGNIFICATION

At 18 Ringer was replaced by arterial blood, and this at 19 by arterial blood to which had been added adrenalin to make up 1:100,000,000. At 20 Ringer was replaced by arterial blood, and this at 21 by the third adrenal specimen. (Reduced to one-half.)

was 72 per cent (cat's blood is usually very rich in serum) so that the blood could not have had a greater concentration at most than 1:400,000,000 of epinephrin. The rate of blood flow during collection of the fourth adrenal specimen was 0.4 gram per minute. The output of epinephrin per minute, accordingly, could not have been more than 0.000001 mgm. per minute for the one adrenal, or 0.000002 mgm. for the two adrenals (0.000001 mgm. per kilo of body weight per minute). This is no more than one-two-hundred and fiftieth of the normal output, as estimated in drawn adrenal blood under our experimental conditions on rabbit intestine and uterus segments, and no more

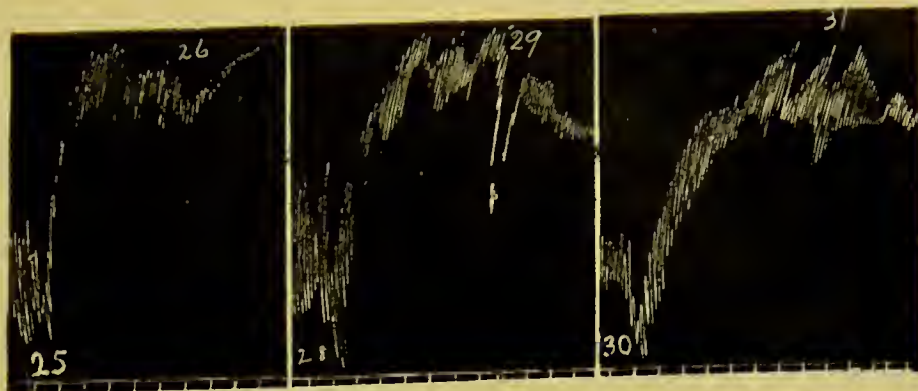


FIG. 14. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURES 12 AND 13

A fresh segment was taken. At 25 Ringer was replaced by serum of arterial blood, and this at 26 by serum of the fourth adrenal specimen. At 28 and 30 Ringer was replaced by arterial blood, and this at 29 and 31 by arterial blood to which adrenalin had been added to make up 1:60,000,000 and 1:100,000,000, respectively. (Reduced to one-half.)

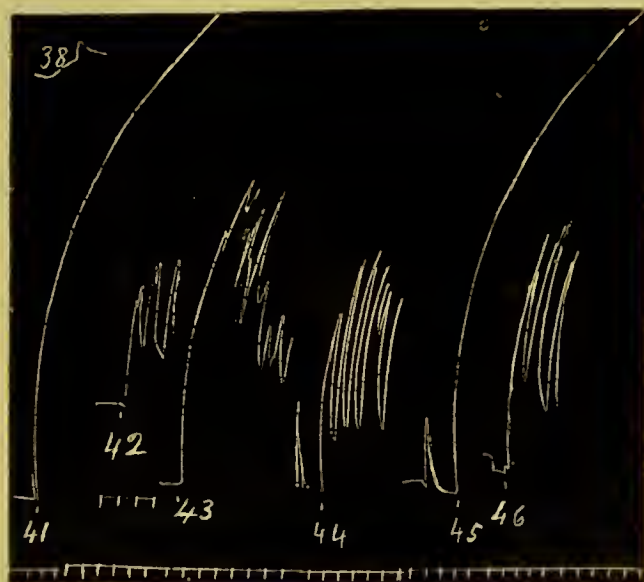


FIG. 15. UTERUS TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 12 to 14

At 38 Ringer was replaced by the second adrenal specimen diluted with an equal volume of Ringer; at 41 by arterial blood to which was added adrenalin 1:200,000,000 (writing point went above drum and stayed up a long time); at 42 by arterial blood, to which was added adrenalin 1:400,000,000. The arterial bloods were diluted with an equal volume of Ringer before adding the adrenalin. At 43 Ringer was replaced by the serum of the fourth adrenal specimen undiluted; at 44 by arterial blood to which was added adrenalin (1:300,000,000); at 44 by adrenalin, 1:300,000,000; at 45 by adrenalin, 1:200,000,000 (writing point went above drum and stayed up long); at 46 by adrenalin, 1:300,000,000. The adrenalin was added in each case to arterial blood previously diluted with an equal volume of Ringer. (Reduced to two-fifths.)

than one-five-hundredth of the normal output, as estimated by eye reactions.

Experiment 9. Condensed protocol. Cat. Weight 3.87 kgm. Three weeks before the experiment the right adrenal gland was excised and the left semilunar ganglion extirpated. The lumbar sympathetic chain was also cut and one lumbar ganglion below the diaphragm excised. The left superior cervical ganglion was excised 6 days before the experiment.

11.30 a.m. Urethane 6 grams by stomach tube.

1.00 p.m. Tracheal cannula inserted and jugular vein blood obtained.

1.20 p.m. to 1.45 p.m. Cava pocket made, tying off renal, coeliac, mesenteric arteries and abdominal aorta.

1.50 p.m. Pocket experiment. 1 minute, 35 seconds. No eye reactions.

1.52 p.m. Pocket experiment. 3 minutes. No eye reactions.

The following specimens of adrenal blood were then collected:

NUMBER OF ADRENAL SPECIMENS	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE
	<i>grams</i>	<i>minutes</i>	<i>seconds</i>	<i>grams</i>
1	2.2	1	25	1.5
2	5.5	4		1.4
3	4.7	4		1.2
4	5.0	5		1.0
5	5.3	8		0.66
6	5.8	10	30	0.55

Obtained blood from carotid.

Right adrenal weighed 0.320 gram and contained 0.33 mgm. epinephrin. Left adrenal weighed 0.316 gram and contained 0.32 mgm. epinephrin.

The first adrenal specimen contained 55 per cent serum (hematocrite) an unusually small proportion of serum for a cat.

The eye reactions in experiment 9 were negative. Some of the tracings of the segment tests are reproduced in figures 16 to 19. None of the samples of adrenal blood gave any reactions indicating the presence of epinephrin, either with intestine or uterus, although the intestine could detect 1:300,000,000 adrenalin (fig. 17, observation 24). A concentration of 1:200,000,000

caused a marked effect on the intestine (observation 22). With the uterus employed it was proved that a concentration of 1:300,000,000 was quite easily detectable (tracing not reproduced), and even 1:500,000,000 (fig. 19, observation 49) could be detected.

In figure 16 it is shown that (in dilution 1:2), even the sixth adrenal blood specimen, which normally would be relatively

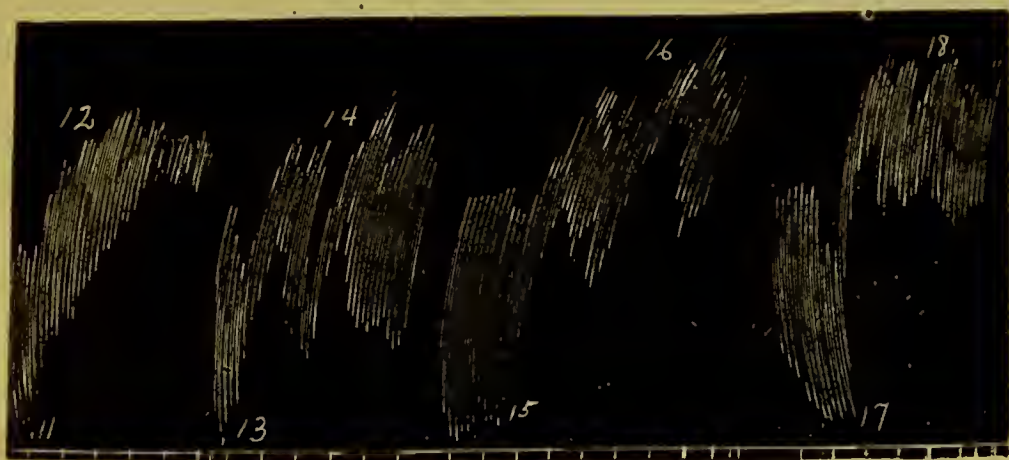


FIG. 16. INTESTINE TRACINGS. BLOODS FROM A CAT WITH RIGHT ADRENAL EXCISED, LEFT SEMILUNAR GANGLION EXTIRPATED AND LUMBAR SYMPATHETIC CHAIN SEVERED THREE WEEKS BEFORE THE EXPERIMENT

Anesthetized with urethane. At 11 Ringer was replaced by jugular blood and this at 12 by the sixth adrenal specimen. At 13 Ringer was replaced by jugular blood, and this at 14 by jugular blood to which was added adrenalin 1:50,000,000. At 15 Ringer was replaced by jugular blood and this at 16 by the fourth adrenal specimen. At 17 Ringer was replaced by jugular blood and this at 18 by jugular blood to which was added adrenalin 1:100,000,000. All the bloods, including the adrenalin bloods after being made up to the concentrations mentioned, were diluted with two volumes of Ringer before application to the segment. (Reduced to one-half.)

rich in epinephrin, owing to the small blood flow at the time of collection, caused no inhibition whatever (observation 12). The same was true of the fourth adrenal blood specimen (observation 16). The intestine segment gave a good reaction with adrenalin blood (1:50,000,000) (observation 14), similarly diluted, and a distinct reaction with adrenalin blood (1:100,000,000) (observation 18).

In figure 17, are displayed the results of some of the tests on intestine segments with undiluted blood. The sixth adrenal specimen gave no definite inhibition (observation 20). Indifferent blood to which adrenalin had been added to make up a concentration of 1:200,000,000, gave a good reaction (observation 22), and even a concentration of 1:300,000,000 caused distinct inhibition (observation 24). Even the serum (of the fifth adrenal specimen) caused no inhibition, but a considerable increase of tone of the intestine segment (fig. 18, observation 33). Adrena-

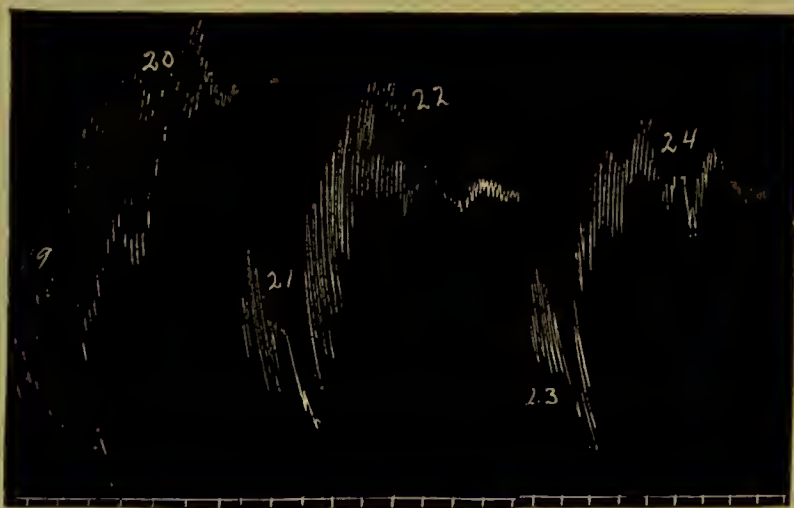


FIG. 17. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURE 16

At 19 Ringer was replaced by indifferent (arterial) blood, and this at 20 by the sixth adrenal specimen. At 21 and 23 Ringer was replaced by arterial blood, and this at 22 and 24 by arterial blood to which had been added adrenalin to make up 1:200,000,000 and 1:300,000,000, respectively. (Reduced to one-half.)

lin in indifferent serum in concentration 1:300,000,000 produced a very different effect on the intestine (observation 35), and one which must be interpreted as an adrenalin action, that is to say, instead of a decided increase of tone, a preliminary slight inhibition followed by a recovery scarcely above the initial level.

The third adrenal blood specimen (diluted with 2 volumes Ringer) caused also a rise of tone, without inhibition (observations 28). All the other adrenal specimens were tested, but

none of them, not even the first, or so-called "manipulation" specimen, gave any positive effect.

The uterus tests confirmed the negative results obtained with the intestine. Specimens of the tracings are reproduced in figure 19. The third undiluted adrenal blood sample (observation 50), gave a somewhat smaller increase of tone than adrenalin

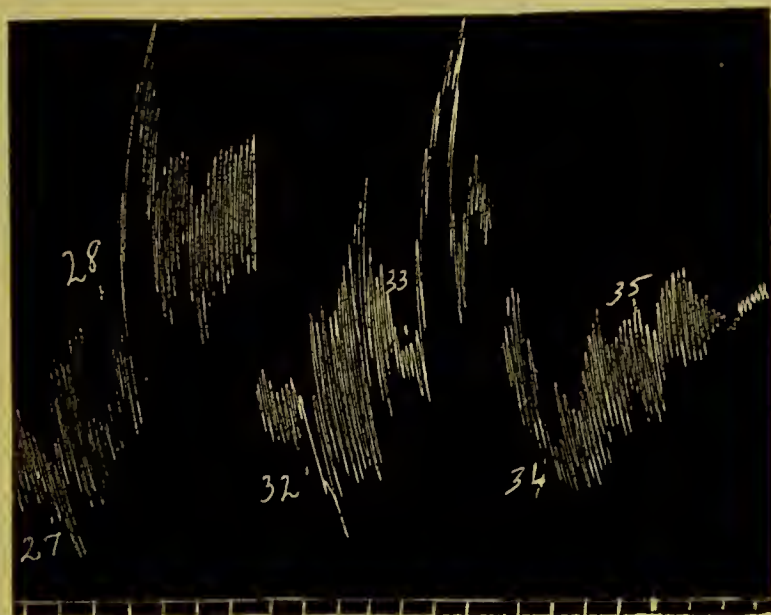


FIG. 18. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 16 AND 17

At 27 Ringer was replaced by arterial blood and this at 28 by the third adrenal specimen, the bloods being diluted with two volumes Ringer before application to the segment. At 32 Ringer was replaced by serum of arterial blood and this at 33 by serum of the fifth adrenal specimen. At 34 Ringer was replaced by serum of arterial blood and this at 35 by serum of arterial blood to which adrenalin had been added to make up 1:300,000,000. All the sera were undiluted. (Reduced to two-thirds.)

added to the second adrenal specimen to make up a concentration of 1:500,000,000. The serum of the fifth adrenal blood sample (observation 51), produced a rise of tone of about the same size as this adrenalin blood, probably a little less. No difference between the indifferent bloods and the sixth adrenal blood specimen was brought out by diluting them to the same degree (observations 38 to 40).

There is evidence, then, from the intestine tests that the adrenal blood certainly did not contain 1:300,000,000 epinephrin, and that even the serum in all probability did not contain 1:300,000,000. The uterus tests bring the possible concentration still lower, probably to 1:500,000,000 for the serum. But even taking 1:400,000,000 as the concentration which the serum could not have exceeded, we get for the blood (of the fifth adrenal specimen) a concentration of no more at most than 1:700,000,000. The rate of flow during the collection of this specimen was 0.65

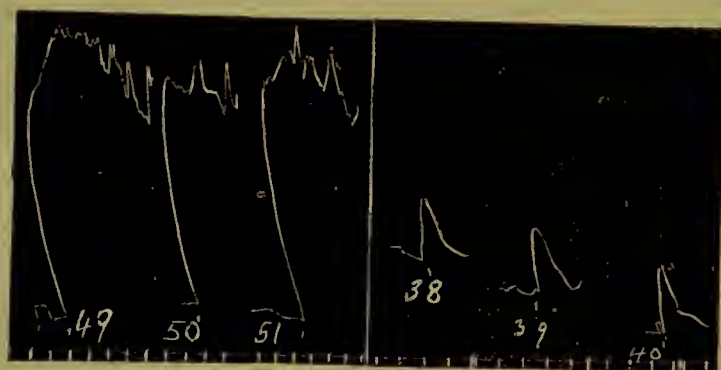


FIG. 19. UTERUS TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 16 TO 18

At 38 Ringer was replaced by arterial blood; at 39 by jugular blood; at 40 by the sixth adrenal blood specimen. The bloods were diluted with two volumes Ringer. At 49 Ringer was replaced by blood of the second adrenal specimen to which had been added adrenalin to make up 1:500,000,000. At 50 Ringer was replaced by the third adrenal specimen; at 51 by serum of the fifth adrenal specimen. In observations 49 to 51 there was no dilution before application to the segment. (Reduced to one-half.)

gram (say 0.6 cc.) per minute. The rate of liberation of epinephrin accordingly could not have been as much as 0.0000009 mgm. per minute for the one adrenal (or 0.0000004 mgm. per kilogram of body weight per minute for two adrenals), i.e., not one-five-hundredth of the normal output, as estimated on drawn adrenal blood by rabbit segments, or one-thousandth of the normal output, under similar experimental conditions, as estimated by the eye reactions.

Results such as those of experiments 8 and 9 indicate that no epinephrin is normally liberated from the adrenals, except through

nerves. It must be remembered that the experimental conditions under which the blood samples were obtained are such as are usually considered to be most favorable for increasing the epinephrin output (anaesthesia, trauma, etc.)

Another conclusion which seems to follow is that the epinephrin normally liberated from the adrenals is not indispensable for life and health. For these cats had not suffered in any respect from the lack of it, so far as could be made out. If it be said that quantities and concentrations of epinephrin smaller than can be detected by the test objects employed, may still, for all we know, play an important part in the organism, the possibility may be conceded. But we do not know any functions which can be demonstrably affected by epinephrin in concentrations of 1:70 thousand millions (the adrenal blood is diluted at least one hundred times in the right heart). And reactions which can be demonstrated are the only reactions which can be studied.

No account is here taken of the possibility that the sporadic chromaffin tissue may discharge a certain amount of epinephrin, since although it has been shown to contain that substance (4) nothing is known as to its liberation. Accessory adrenals were always looked for *post mortem*, but were not present in any of our cats. In any case, accessory adrenals consist of cortical, not of medullary tissue.

It is scarcely necessary to say that our results do not show that no important or indispensable substance continues to be given off by the adrenals or even by the adrenal medulla after section of their nerves. On the contrary, as the adrenals are indispensable to life it must be assumed that the most important of their functions can be carried on through the blood or lymph in the absence of the nerves which control the liberation of epinephrin. The objection that the rate of liberation was not determined in these cats before section of the nerves and that it might have been exceptionally small, has no weight. For we do not find very wide variations in the output under our experimental conditions (table 2), and we never encounter a cat with intact adrenal nerves whose adrenal vein blood does not con-

tain epinephrin in concentration far above the limit of detectability with test objects of normal sensitiveness.

The concentration in dog's adrenal blood is usually considerably less than in the cat, probably due largely, if not entirely, to the greater rate of blood flow. It is for this reason easier to miss detecting epinephrin in dog's adrenal vein blood than in cat's, especially if the blood flow has not slackened considerably during collection of a series of samples and if rather insensitive segments happen to be employed. This is undoubtedly the reason why in previous experiments made by one of us (5) a negative result was sometimes obtained in blood from the dog's adrenal veins, with the nerves intact. Occasionally also in the previous experiments on dogs there was some admixture of the adrenal blood with blood from the transverse lumbar vein, which would still further reduce the epinephrin content. It is not very uncommon in the dog, to find one adrenal vein, especially the left, opening into the renal vein, and if this is not recognized in tying off the cava pocket, the adrenal vein may easily be occluded. Although this would not affect the concentration of epinephrin in the blood collected, it would diminish the apparent rate of liberation by half.

In three experiments, the cats were allowed to live for such a time (15 weeks), as to permit at least the possibility of some regeneration of the fibers. These animals differed in no respect in their behavior and state of health from the cats which were allowed to survive for shorter periods.

Experiment 10. Condensed protocol. Cat. Weight 2.06 kgm. at the first operation. The left adrenal gland was excised and the nerves coming to the right semilunar ganglion severed 15 weeks before the experiment. The left superior cervical ganglion was excised one week before the experiment. Body weight 2.63 kgm. at the time of the experiment. Condition good.

10.40 a.m. Ether; cava pocket tied off, renal, coeliac, mesenteric arteries and abdominal aorta below the renals being ligated.

11.10 a.m. Pocket experiment 52 seconds. No eye reactions.

- 11.15 a.m. Pocket experiment 1 minute, 32 seconds. No eye reactions. About 10 cc. blood was obtained from the jugular vein.
- 11.30 a.m. Pocket experiment 1 minute, 30 seconds. No eye reactions.
- 11.35 a.m. Pocket experiment 1 minute, 50 seconds. No eye reactions. Tracheal cannula inserted, also cannula in lower end of pocket, in iliac vein.
- 11.52 a.m. Adrenal blood collected from pocket. Flow very slow, about 5 cc. in 27 minutes. Obtained blood from right heart. Capacity of pocket, slackly filled, 0.39 gram. Left adrenal weighed 0.346 gram and contained 0.22 mgm. epinephrin. Right adrenal weighed 0.330 grams and contained 0.22 mgm. epinephrin. The bloods were centrifuged and the serums tested.

The eye reactions for epinephrin in the adrenal blood were negative. With the uterus segments (fig. 20), no evidence of the presence of epinephrin even in the serum of the adrenal vein blood was obtained, although the blood flow during the collection of the specimen was slow. The adrenal serum (diluted with 5 volumes Ringer), caused scarcely as great an increase of tone as the jugular serum similarly diluted (observations 19 and 20). With a smaller degree of dilution (1:3), adrenal serum was distinctly inferior to jugular in tone-increasing power. The rise caused by the undiluted adrenal serum (observation 31), on a different uterus segment, was much smaller than that due to an adrenalin solution (1:30,000,000) in an indifferent serum (diluted with 3 volumes Ringer) (observation 30). There was evidence that the rise of tone caused by the adrenalin solution was maximal for this segment. So that the uterus could undoubtedly have detected a far smaller concentration of adrenalin than 1:30,000,000. The indifferent serum in which the adrenalin solution was made up (diluted with 3 volumes Ringer), caused a somewhat greater rise than the undiluted adrenal serum.

The great difference in tone-increasing power between the adrenal and the indifferent sera in this experiment suggests that the former contained some substance which caused inhibition

of uterus tone. It is known that such a substance is sometimes present in venous blood, during asphyxial conditions. For this reason it is certain that the inhibitory effects obtained on the intestine segment were not due, at least wholly, to epinephrin. It is not often that this circumstance complicates the estimation of the concentration of epinephrin by the segments (this experiment, indeed, is the only instance in the series recorded in this paper). But it can only be controlled by using both uterus and

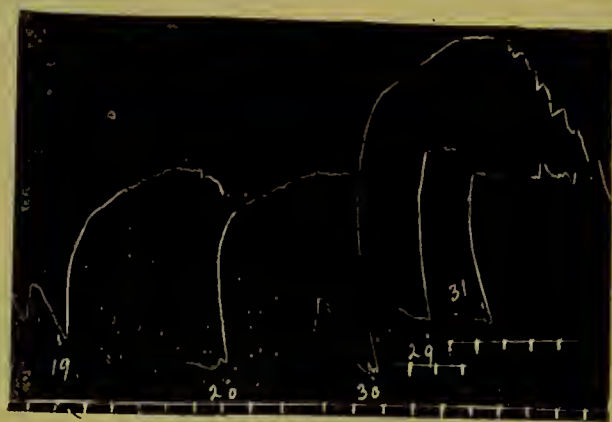


FIG. 20. UTERUS TRACINGS. BLOODS FROM CAT WITH LEFT ADRENAL EXCISED AND NERVE CONNECTIONS OF RIGHT SEMILUNAR GANGLION CUT FIFTEEN WEEKS BEFORE EXPERIMENT

Anesthetized with urethane. At 19 Ringer was replaced by serum of adrenal blood (a small sample slowly collected), at 20 by serum of jugular blood; both diluted with five volumes Ringer. At 29 (with another segment) Ringer was replaced by serum of a dog diluted with three volumes Ringer; at 30 by dog serum to which adrenalin had been added to make up 1:10,000,000, the adrenalin serum being then diluted with three volumes Ringer before application to the segment. At 31 Ringer was replaced by undiluted serum of the cat's adrenal blood. (Reduced to one-half.)

intestine segments for the tests. Even if the inhibition of the intestine (figure 21) caused by the adrenal serum (observation 6) were entirely due to epinephrin, the concentration in the serum must have been much less than 1:10,000,000 (observation 14), and in the blood much less than 1:17,000,000. If we take the concentration in the blood even at 1:20,000,000 this would correspond to an epinephrin output of no more than 0.00001 mgm. per minute for one adrenal (or 0.000007 mgm. per kilo-

gram of body weight per minute for the two adrenals). This is not more than one-thirtieth to one-fortieth of the normal output as estimated on drawn adrenal blood on rabbit segments, or one-hundredth of the normal as estimated by the eye reactions.

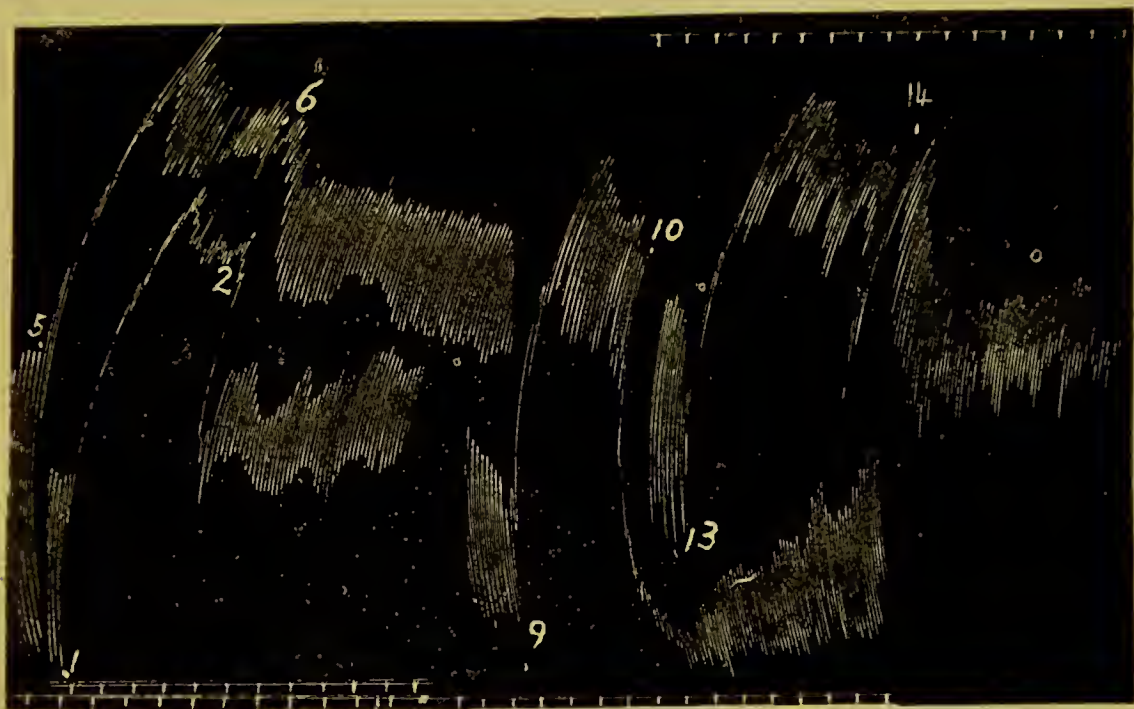


FIG. 21. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURE 20

At 1 Ringer was replaced by serum of jugular blood and this at 2 by serum of adrenal blood. The sera in observations 1 and 2 were undiluted. At 5 Ringer was replaced by jugular serum and this at 6 by adrenal serum, both diluted with an equal volume of Ringer. At 9 Ringer was replaced by dog serum diluted with an equal volume of Ringer and this at 10 by the same serum made up to 1:3,000,000 with adrenalin, and then diluted with an equal volume of Ringer before application to the segment. At 13 Ringer was replaced by jugular serum and this at 14 by jugular serum to which adrenalin had been added to make up 1:10,000,000. Both jugular serum and adrenalin serum were diluted with an equal volume of Ringer before application to the segment. (Reduced to one-half.)

Experiment 11. Condensed protocol. Cat. Weight 3.06 kgm. at the time of the operation, 4.14 kgm. at the time of the experiment. The right adrenal gland was excised and the nerves coming to the left semilunar ganglion cut 15 weeks before the experiment. The left superior cervical ganglion was excised 8 days before the experiment. Condition excellent.

- 11.30 a.m. 4 grams urethane.
- 1.00 p.m. Inserted tracheal and jugular cannulae. Obtained sample of jugular blood.
- 1.20 p.m. Made cava pocket, with cannula in a renal vein.
- 2.05 p.m. Pocket experiment 2 minutes, 10 seconds. Dubious reaction of left pupil and nictitating.
- 2.15 p.m. Pocket experiment 4 minutes. Slight dilatation of pupil in 16 to 18 seconds; retraction of nictitating about 10 seconds later.
- 2.30 p.m. Collected blood from pocket through cannula. Poor flow. About 2 cc. of adrenal blood collected in 20 minutes.
- 2.55 p.m. Isolated left sympathetic in thorax on a loose ligature.
- 3.00 p.m. Pocket experiment, 2 minutes, 35 seconds. Small dilatation of pupil in 26 seconds, nictitating following 10 to 12 seconds later.
- 3.06 p.m. Pocket experiment 3 minutes, 30 seconds. Small pupil dilatation in 16 to 18 seconds, and nictitating about 12 seconds later.
- 3.13 p.m. Tied and cut left sympathetic in thorax.
- 3.15 p.m. Pocket experiment 4 minutes. Slight retraction of nictitating in 32 seconds. Pupil reaction doubtful.
- 3.21 p.m. Pocket experiment, 5 minutes. Very slight eye reactions.
- 3.29 p.m. Peripheral end of left sympathetic stimulated for 25 seconds with pocket open. Slight instantaneous dilatation of pupil. No other pupil reaction.
- 3.35 p.m. Stimulated left sympathetic 54 seconds. No eye reactions.
- 3.37 p.m. Pocket experiment 4 minutes. No eye reactions.
- 3.51 p.m. Pocket experiment with stimulation of left sympathetic 4 minutes. Very slight but definite pupil dilatation in 44 seconds after release of the pocket, very slight retraction of nictitating following about 9 seconds later. Inserted cannula in lower end of pocket and obtained a small sample of adrenal blood. Right adrenal weighed 0.186 gram and contained 0.23 mgm. epinephrin. Left adrenal weighed 0.190 gram and contained 0.12 mgm. epinephrin.

In experiment 11, slight but positive eye reactions were obtained, indicating a much reduced, but still detectable liberation

of epinephrin. The first adrenal blood sample was found, by the segment tests, to contain approximately 1:5,000,000 epinephrin. This concentration would be rather low for a cat, even with a normal blood flow, and is very low for the small flow when the sample was collected. The output per minute could not have been more than 0.00002 mgm. for the one adrenal (or 0.00001 mgm. per kilogram of body weight per minute for the two adrenals). This is not more than one-twenty-fifth of the normal output as estimated on drawn adrenal blood by rabbit segments, and no more than one-fiftieth or one-sixtieth of the normal as estimated by the eye reactions. It is worthy of note that the section of the left sympathetic in the thorax diminished the already slight eye reactions and stimulation of the nerve increased them slightly, but definitely. In this cat, then, some of the secretory fibers in the sympathetic were capable at this time of conduction. It is, of course, impossible to say whether these were regenerated fibers or fibers which had escaped section when the nerves coming to the semilunar ganglion were cut.

Experiment 12. Condensed protocol. Cat. Weight at first operation 2.09 kgm., at the time of experiment 3.635 kgm. The right adrenal was excised and the nerves coming to the left semilunar ganglion cut 15 weeks before the experiment. The left superior cervical ganglion was excised 6 days before the experiment. Condition excellent.

11.00 a.m. Urethane 4 grams.

1.30 p.m. Tracheal cannula inserted. Pocket made.

2.10 p.m. Pocket experiment, 1 minute, 30 seconds occlusion. Good pupil reaction in 12 seconds after release of pocket, nictitating 4 seconds later.

2.27 p.m. Pocket experiment, 2 minutes. Pupil reaction in 14 seconds, nictitating 10 seconds later.

2.31 p.m. Pocket experiment, 2 minutes. Good pupil reaction in 14 seconds, nictitating 10 seconds later.

Collected adrenal blood through cannula in lower end of pocket. Flow very slow. Poor circulation. About 3 cc. collected in 26 minutes. Isolated left sympathetic in thorax on loose ligature.

3.25 p.m. Pocket experiment, 2 minutes. Slight pupil and nictitating reactions.

- 3.32 p.m. Pocket experiment, 2 minutes, 30 seconds. Pupil reaction in 28 seconds, nictitating 6 seconds later. Tied and cut left sympathetic in the thorax.
- 3.40 p.m. Pocket experiment, 3 minutes. Slight pupil reaction in 24.5 seconds. No nictitating. Cut right sympathetic in thorax.
- 3.50 p.m. Pocket experiment, 3 minutes. Slight pupil reaction in 26 seconds. No nictitating.
- 3.55 p.m. Pocket experiment, with stimulation of left sympathetic 3 minutes. About the same reaction as at 3.50 (in 26 seconds).
- 4.00 p.m. Pocket experiment, 3 minutes. Slight pupil reaction in 30 seconds. No nictitating.
- 4.05 p.m. Pocket experiment, with stimulation of left sympathetic, 3 minutes. Smaller pupil reaction than at 4.00, in 36 seconds. Blood obtained from abdominal aorta, with pocket still clipped off. Right adrenal weighed 0.150 gram and contained 0.16 mgm. epinephrin. Left adrenal weighed 0.164 gram and contained 0.09 mgm. epinephrin.

The eye reactions were positive in experiment 12. They were not diminished by section of the sympathetics in the thorax, nor were they increased by stimulation of the left sympathetic. In this cat, there was no evidence that any of the secretory fibers in the sympathetic were at this stage capable of conduction. The intestine segment tests showed that the adrenal vein blood contained much less than 1:3,000,000 epinephrin (fig. 22), and not far from 1:6,000,000 (fig. 23). The uterus tests confirmed this (fig. 24). For instance, the adrenal blood diluted with 5 volumes Ringer (observation 19), gave a somewhat smaller increase of tone than 1:6,000,000 adrenalin made up in indifferent blood and similarly diluted. The flow during collection of the adrenal specimen was slow, doubtless considerably slower than during the eye tests. The output of epinephrin per minute was 0.00002 mgm. for the one adrenal (or 0.00001 mgm. per kilogram of body weight per minute for the two adrenals). This is not more than one-twentieth of the normal output, as estimated on shed adrenal blood by

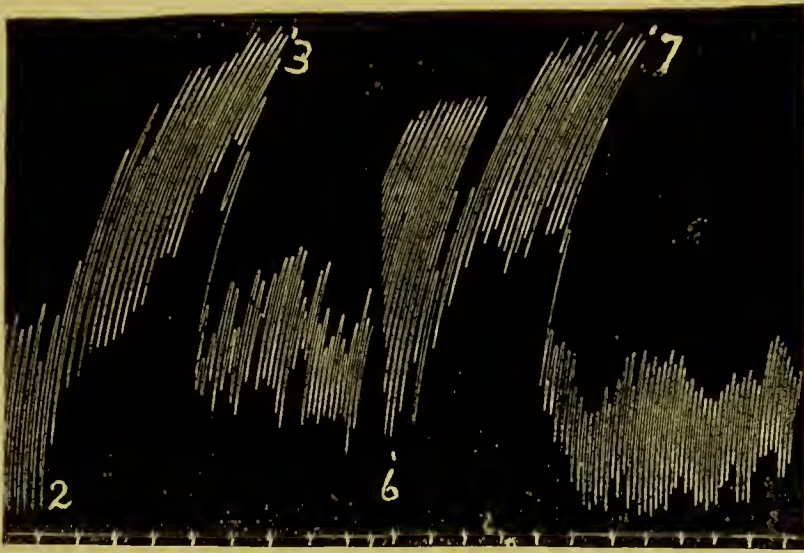


FIG. 22. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL EXCISED AND NERVE CONNECTIONS OF LEFT SEMILUNAR GANGLION CUT FIFTEEN WEEKS BEFORE EXPERIMENT

Anesthetized with urethane. At 2 Ringer was replaced by arterial blood, and this at 3 by adrenal blood (a small sample very slowly collected). At 6 Ringer was replaced by arterial blood and this at 7 by arterial blood containing, 1:3,000,000 adrenalin. (Reduced to two-thirds.)

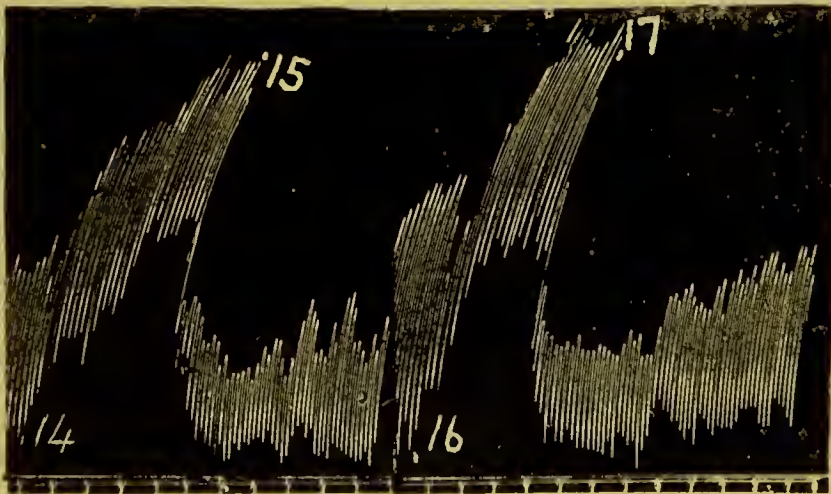


FIG. 23. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURE 22

At 14 Ringer was replaced by arterial blood and this at 15 by adrenal blood, both diluted with an equal volume of Ringer. At 16 Ringer was replaced by arterial blood diluted with one volume Ringer, and this at 17 by arterial blood to which adrenalin had been added to make up 1:6,000,000, the adrenalin blood being diluted with one volume Ringer before application to the segment. (Reduced to two-thirds.)

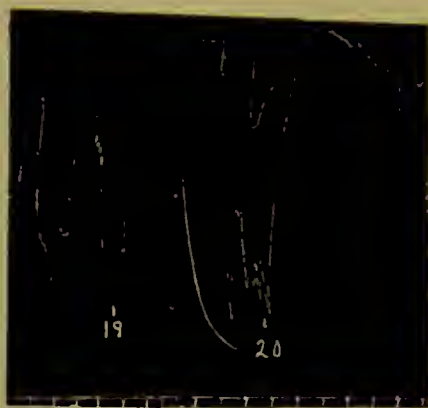


FIG. 24. UTERUS TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 22 AND 23

At 19 Ringer was replaced by adrenal blood diluted with five volumes Ringer; at 20 by arterial blood to which had been added adrenalin to make up 1: 6,000,000, the adrenalin blood being then diluted with five volumes Ringer before application to the segment. (Reduced to one-half.)

rabbit intestine segment tests, and not more than one-fortieth of the output, as estimated by eye reactions.

SUMMARY

1. We showed in a previous paper, by the blood pressure and eye reactions, that after section of the nerve supply of the adrenal no demonstrable liberation of epinephrin was present in cats as long as five weeks after the nerve section.

2. As it is easier to detect very small concentrations of epinephrin by the rabbit intestine and uterus segments, we have made a series of survival experiments in cats in which these tests were used to supplement the eye reactions. In all the animals one adrenal was excised and the nerves of the other cut.

In a cat tested two weeks after the operation, it was shown that the adrenal blood serum could not have contained 1: 300,000,000, or the blood 1: 400,000,000 of epinephrin; and that the rate of liberation of epinephrin could not have been at most 0.000001 mgm. per minute for one adrenal. In another cat three weeks after the operation the serum of the adrenal blood was proved to contain less than 1: 400,000,000 and the blood

less than 1:700,000,000 epinephrin. The output of epinephrin per minute could not have been as much as 0.0000009 mgm. per minute, for one adrenal. The segments used for the tests in these experiments were extremely sensitive, and the limits of adrenalin concentrations which could be detected with certainty were carefully determined. The eye reactions were negative. In these two cats the rate of liberation of epinephrin, if any liberation whatever was going on, must have been several hundred times less than the rate in normal animals under the same experimental conditions.

It is scarcely necessary to point out that experiments yielding completely negative results indicating the absence of epinephrin with very sensitive test objects are much more important for the questions studied than experiments in which small amounts of epinephrin can still be detected. For it is impossible to be certain that when a little epinephrin is found some of the fibers concerned in the liberation may not have escaped section.

3. Since these animals had completely recovered from the operation and behaved in every way like normal animals, it must be concluded that the liberation of epinephrin from the adrenals is not indispensable for life or health, unless indeed the necessary quantity is, even in the adrenal vein blood, below the limits of detection by the methods used. The epinephrin in the adrenal blood is diluted enormously (probably at least one-hundred times) in the right heart; so that in these cats the concentration in the arterial blood could not, at most have reached 1:40,000,000,000 and 1:70,000,000,000, respectively.

If the liberation of epinephrin is totally abolished by division, in the dorsal cord, of the path concerned in it, as our experiments⁴ on the Relation of the Spinal Cord to the Spontaneous Liberation of Epinephrin indicate, this corroborates the conclusion that epinephrin is not indispensable, since numerous animals and men have long survived such lesions.

4. The experiments indicate that the entire liberation of epinephrin from the adrenals is controlled by nerves.

⁴ Proc. Soc. Exp. Biol. and Med., April 18, 1917; Jour. of Exper. Med., xxvi, 1917.

5. In some of the other cats the residual output of epinephrin was so small that it was doubtful whether it was being liberated at all in detectable amount. In all, the rate of liberation, even where a definite output could still be detected, was reduced to a small fraction of the normal.

6. In a number of acute experiments on cats and dogs, the reduction in the output of epinephrin after section of the various possible nerve paths to the adrenals was studied. In all, epinephrin was still found in detectable amount in the blood coming from the adrenals, although the rate of liberation was reduced to a small fraction of the initial amount.

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- (2) STEWART AND ROGOFF: *Journ. Pharm. Exp. Ther.*, 1916, viii, 205.
- (3) STEWART AND ROGOFF: *Journ. Pharm. Exp. Ther.*, 1917, ix, 393.
- (4) FULK AND MACLEOD: *Amer. Journ. of Physiol.*, 1916, xl, 21.
- (5) STEWART, G. N.: *Journ. Exp. Med.*, 1912, xv, 547.

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Relation of the spinal cord to the spontaneous liberation of epinephrin.

By G. N. STEWART and J. M. ROGOFF.

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1. In acute experiments on cats, anesthetized with urethane, section of the spinal cord in the cervical region caused no demonstrable diminution in the rate of liberation of epinephrin from the adrenals as tested by allowing the adrenal blood collected in a cava pocket to elicit eye-reactions (after preliminary excision of the superior cervical ganglion). Four such experiments were made. The cord section in one of these was between the third and fourth cervical vertebræ, in another opposite the body of the fourth cervical vertebrae, in a third just below the body of the fifth cervical vertebra, and in a fourth cat between the fifth and sixth cervical vertebræ. In the second of these animals the blood was collected from the adrenal vein before and after section of the cord. The epinephrin assay (by rabbit intestine segments) gave the same output of epinephrin per minute after as before the section. The blood flow from the adrenals was much slower after the section, but was correspondingly richer in epinephrin.

2. In two survival experiments the cord was cut in the cervical region (in one just above the body of the last cervical vertebra, in the other at the level of the body of that vertebra). In the first cat, the superior cervical ganglion had been previously excised. Two days after the cord section adrenal blood was tested by the cava pocket method and gave good eye reactions, indicating a fairly good liberation of epinephrin (at least 0.0004 mgm. per minute). In the second experiment blood was drawn three days after the cord section and tested on rabbit intestine and uterus segments. Good concentrations of epinephrin were found (1 : 1,500,000 in the fourth adrenal sample, more than 1 : 2,500,000 and less than 1 : 1,500,000 in the second adrenal sample). Al-

though the blood flow was small (0.3 gm. per minute for the second sample) a substantial liberation of epinephrin was demonstrated.

3. In one acute experiment, the spinal cord was cut between the fifth and sixth cervical vertebræ. The pupil reaction was not noticeably diminished. The cord was then severed between the fourth and fifth dorsal vertebræ. The pupil reaction could no longer be obtained. When the cord was now stimulated with induction shocks between the fifth and sixth dorsal vertebræ, and blood collected in the cava pocket during stimulation, good eye reactions were elicited on releasing the pocket.

4. In one survival experiment, the cord was cut between the fifth and sixth dorsal vertebræ. Three days afterwards the adrenal vein blood was tested by the cava pocket method but no eye reactions could be obtained. The pupil gave a good reaction with 0.2 c.c. of 1 : 500,000 adrenalin. On intestine segments negative results were obtained with adrenal blood, although a concentration of 1 : 60,000,000 adrenalin in indifferent blood caused a distinct effect. It was shown that the adrenal vein blood could not have contained 1 : 100 000,000, and that the discharge of epinephrin per minute could not have been at most 0.000003 mgm., that is, not one hundredth of the output to be expected in a normal cat under the experimental conditions. It was not demonstrated that any epinephrin was present.

5. In three of the cats with the cervical cord transected (1 survival, and 2 acute experiments), the effect on the eye reactions of severing nerves containing the fibers concerned in the liberation of epinephrin (sympathetics and splanchnics in thorax, splanchnics in abdomen, and other nerves coming to semilunar ganglion, lumbar sympathetic chain) was studied. The eye reactions still obtainable from the adrenal blood after the cervical section were greatly weakened or abolished after the division of those nerves.

6. It seems to follow from these observations, that liberation of epinephrin from the adrenals is still sustained after division of the cord in the cervical region at the levels mentioned, and that this liberation takes place through the splanchnic and other nerves known to be concerned when the spinal cord is still connected with the brain. The contrast between the epinephrin output when the cervical cord has been divided and when the dorsal cord has been divided at the levels mentioned is very great.

THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS.*

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PLATES 44 TO 47.

(Received for publication, May 10, 1917.)

The demonstration of the complete dependence of the spontaneous liberation of epinephrin from the adrenals upon the integrity of their nerve supply naturally raises the question where the central mechanism which sustains this secretion is situated. We are not aware of the existence of evidence upon this point. Elliott's statement¹ that exhaustion of the store of epinephrin in the adrenals by electrical excitation of afferent nerves does not occur if the cord is transected anywhere below the level of the vasomotor center in the medulla oblongata, but does occur when transection of the brain-stem is made just above the anterior corpora quadrigemina, has no direct bearing on the question. For it has not been proved that the exhaustion of the store is due entirely or mainly to increase in the rate of liberation of epinephrin, on which Elliott made no observations. A change in the amount of the store of epinephrin would merely show that some alteration had occurred in the relation between the rate of formation and the rate of discharge of the epinephrin. Nor would such observations even if they were accepted as proving an increase in the liberation brought about reflexly through a center in the bulb or higher up, give any indication whether the steady spontaneous liberation of epinephrin is sustained from a center at this level. Absence of effect

* A note on this work was published in the *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 143.

¹ Elliott, T. R., *J. Physiol.*, 1912, xliv, 407.

ERRATUM.

On page 616, Vol. XXVI, No. 5, November 1, 1917, foot-note 2, for *Marine, D., and Rogoff, J. M., J. Pharm. and Exp. Therap.*, 1916-17, ix, 1, read *Stewart, G. N., and Rogoff, J. M., J. Pharm. and Exp. Therap.*, 1917, x, 1.

On page 627, foot-note 4, for *Stewart and Rogoff, J. Pharm. and Exp. Therap.*, 1916-17, ix, 479, read *Stewart and Rogoff, J. Pharm. and Exp. Therap.*, 1916, viii, 479.

was approximately sixteen times as great as in the second. In other words, the output of epinephrin (0.0004 mg. per minute) was not altered by the cord section.

It might be objected that the small blood flow during collection of the eighth specimen was not sufficient to prevent partial asphyxia of the adrenals, and that by some direct effect of this condition on the cells of the medulla an abnormal liberation of epinephrin, not mediated through the nervous system, took place. This objection is entirely without weight. For with even smaller flows through adrenals whose innervation has been interrupted no such liberation occurs.² After all, a flow of about 100 gm. of blood per 100 gm. of tissue, although small for the adrenal, is scarcely a starvation allowance.

It might much more plausibly be argued that the calculated output of epinephrin for the eighth specimen is likely to be less than the true rate after section of the cord, provided that the blood flow had not diminished. For it is quite unusual to find under any circumstances in adrenal blood collected in a cava pocket a much greater concentration than 1:1,000,000. If, then, with a declining rate of blood flow the maximum possible concentration has once been reached, the rate of liberation calculated for smaller flows will be less than the gland is capable of sustaining under the given experimental conditions with a more copious flow of blood. However, it is only when the calculated rate of liberation for the smaller flow is much less than for the larger flow, that any such question could arise. When with a relatively small blood flow the calculated output of epinephrin per minute is as great as with a larger flow all our experience goes to show that the rate of output calculated from the concentration and the blood flow can be legitimately compared for a wide range of blood flows lying above this smallest flow.

In the next experiment, the cord was transected at a slightly higher level between the third and fourth cervical vertebræ, through the fourth segment just above the origins of the fourth pair of cervical nerves, as shown at autopsy. The spontaneous liberation of epinephrin was studied by means of the eye reactions, the superior

² Stewart, G. N., and Rogoff, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 145; Marine, D., and Rogoff, J. M., *J. Pharm. and Exp. Therap.*, 1916-17, ix, 1.

cervical ganglion having been previously excised. The eye reactions were employed because they afforded a convenient means of investigating the effects of section of the nerves going to the adrenals upon the spontaneous liberation after section of the cervical cord.

Experiment 2. Condensed Protocol.—Cat; weight 2.19 kilos. Left superior cervical ganglion excised 6 days before the experiment.

9.30 a.m. 4 gm. of urethane given by stomach tube.

10.30 a.m. Tracheal cannula inserted and cervical cord exposed between the third and fourth vertebræ. Cava pocket made, abdominal aorta and renal vessels being tied.

11.15 a.m. Started artificial respiration.

Time.		Duration of pocket.	Pupil dilatation.
<i>a.m.</i>			
11.20	Pocket experiment.	2 min., 40 sec.	None.
11.25	" "	5 "	Small; in 40-45 sec.
11.32	" "	4 " 30 "	" " 31 sec.
11.45	Spinal cord cut just above body of fourth cervical vertebra.		
11.46	Pocket experiment.	6 min., 40 sec.	Fair; in 34 sec.
11.55	" "	4 " 15 "	Small; " 36 "
<i>p.m.</i>			
12.02	" "	4 " 15 "	" " 35 "
12.10	Tied intestinal arteries; cut major and minor splanchnics on both sides in abdomen.		
12.15	Pocket experiment.	6 min., 15 sec.	Small; in 38-40 sec.
12.25	" "	6 " 12 "	" " 43 sec.
12.35	Cut both sympathetics in thorax (including splanchnics) below last rib.		
12.40	Pocket experiment.	6 min., 10 sec.	None.
12.50	" "	10 " 30 "	"

The pocket filled very slowly throughout the experiment; the heart was feeble.

In this experiment, as will be seen from the protocol, section of the cord at the level mentioned caused no noteworthy change in the eye reaction, certainly no clear diminution. The circulation was poor both before and after the section. The cava pocket filled slowly and the interval between the release of the pocket and the beginning of

the pupil dilatation was correspondingly long. Yet approximately the same time of collection was required to evoke a reaction of given magnitude before and after section of the cord. After division of the splanchnics in the abdomen and the sympathetic trunks in the thorax no eye reactions could be obtained, even with much longer periods of collection in the pocket; that is, the liberation of epinephrin after section of the cord must have been sustained from some part of the cord below the fourth cervical segment, and through the same nerves as with intact central nervous system.

It may be explained here that even when the cava pocket has been kept closed for so long a period that it is unable to receive any more adrenal vein blood, there is good evidence that the output of epinephrin still goes on into the blood of the adrenal capillaries at an approximately constant rate; and that when the pocket is released the full effect of this epinephrin is exerted in eliciting the eye reactions or increasing the blood pressure, just as if the blood had actually passed into the pocket. So that in the course of an experiment, the reactions evoked when a pocket clipped off for a given length of time is released are substantially of the same magnitude, whether the pocket has been overfilled or underfilled in that time. Of course, the interval after which the reactions occur is greatly influenced by the rate of the circulation, since the epinephrin-containing blood released from the pocket will take longer to reach the reacting structures with a slow than with a rapid blood flow. With a very slow flow, also, the blood collected in a pocket may be so small in amount that it is not promptly or completely passed into the circulation on release of the pocket. It is further to be expected that below a certain rate of flow the function of the adrenal medullary cells, as already suggested, will be interfered with. In that case, the steadiness, for given conditions, in the output of epinephrin per unit of time, which is so strikingly manifested over a wide range in the rate of the blood flow, will no longer be maintained. Our experience shows, however, that this point is not easily reached.

In the next experiment transection was made about two segments lower in the cervical cord, in order to localize more sharply the level of the cord concerned in the spontaneous secretion of epinephrin.

Experiment 3. Condensed Protocol.—Cat; weight 3.175 kilos. Recent parturition. Left superior cervical ganglion excised 1 week before the experiment.

9.45 a.m. 4 gm. of urethane.

10.45 a.m. Tracheal cannula inserted and cervical cord exposed between fifth and sixth vertebræ. Cava pocket prepared; intestinal and renal arteries and abdominal aorta tied.

11.30 a.m. Left pupil wider than right, both nictitating membranes forward; cat breathing quite well, but for uniformity of observations artificial respiration was started.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
<i>a.m.</i>				
11.40	Pocket experiment.	1 min., 30 sec.	Doubtful.	None.
11.42	" "	2 " 30 "	None.	"
11.45	" "	3 " 45 "	"	"
11.50	" "	5 "	"	"
<i>p.m.</i>				
12.05	Pocket experiment.* Cord stimulated with needle electrodes one segment below the exposed part for 3 min.	3 "	"	"
12.15	Left sympathetic and vagus cut in neck.			
12.17	Right sympathetic cut in neck.			
12.20	Pocket experiment.	5 min., 30 sec.	Very good; in 10 sec.	Very good; 10 sec.
12.26	" "	1 "	Good; in 15 sec.	Good; 15 sec.
12.30	" "	2 "	Very good; in 10.8 sec.	Very good; 10.8 sec.
12.37	Spinal cord cut just below body of fifth cervical vertebra.			
12.41	Pocket experiment.	2 min.	Very good; in 15 sec.	Very good; 15 sec.
12.45	" "	1 "	Good; in 18.6 sec.	Good.

* With each period of stimulation both pupils dilated instantaneously, and proportionally to the same extent, but the left pupil still remained wider than the right. After section of both cervical sympathetics, stimulation of the cord still caused dilatation of both pupils. At autopsy it was found that the cord had been cut between the fifth and sixth cervical segments.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
<i>p.m.</i>				
12.50	Major and minor splanchnics in abdomen cut on both sides.			
12.53	Pocket experiment.	2 min.	None.	None.
12.56	" "	5 "	Slight; in 20-25 sec.	"
1.05	Right semilunar ganglion excised; nerves coming to left ganglion cut. Circulation getting feeble.			
1.12	Pocket experiment.	5 min., 30 sec.	None.	None.
1.25	" "	9 " 30 "	Slight; in 60-90 sec.	Slight.
1.47	" "	24 "	Slight; in 40-45 sec.	
2.25	" "	25 "	None.	None.
3.05	" " with massage of adrenals.	15 "	"	"
3.30	Injected 0.5 cc. of 1:1,600,000 adrenalin.		"	"
3.35	Injected 0.5 cc. of 1:270,000 adrenalin.		Slight; in 40-50 sec.	Slight.

In this animal the denervated eye reactions were employed and an interesting preliminary observation was made upon them without some reference to which the first part of the protocol would probably appear as puzzling to the reader as the observations did to us when they were being made. The left superior cervical ganglion had been excised a week before the experiment. The eye reactions ought, therefore, to have been easily obtained in the pocket experiments made between 11.40 and 12.05 before section of the cord. In the numerous observations made by us, we have, apart from this experiment, scarcely ever had a negative result, especially with a duration of occlusion of the pocket as long as 5 minutes, and with the good blood flow and satisfactory filling of the pocket which existed in this cat. It was conceivable, of course, that for some reason the adrenals might have been giving off much less epinephrin than usual; or that the reactions of the iris, etc., might have been unduly depressed by

the anesthetic, although there was nothing in the behavior of the animal to indicate that the urethane, a very uniform anesthetic, as is well known, had affected this animal at all differently from any of the others. Desiring to increase the output of epinephrin to the maximum, we stimulated the cord with needle electrodes, hoping thus to strike the secretory path, but again with a negative result as regards the eye reaction. Even with the collection of adrenal blood in the pocket for as long as 3 minutes during stimulation no eye reactions were obtained. Stimulation of the cord, however, caused immediate dilatation of the pupils of both eyes. This dilatation was still elicited after section of both vago-sympathetics in the neck, and may be attributed to stimulation of afferent fibers in the cord. But the interesting point was that after section of the cervical sympathetics excellent eye reactions were now evoked with collections of adrenal blood in the cava pocket much shorter than those which gave a negative result before division of the nerves. The most probable conclusion would seem to be that some small part of the superior cervical ganglion had escaped excision, and that the innervation of the eye through the cervical sympathetic was not entirely interrupted, although it was impossible to verify this at autopsy on account of scar tissue. The change in the sensitive structures of the iris and nictitating membrane, on which the increased power of reaction to adrenalin depends, must be assumed to have developed as usual after removal of the ganglion, although prevented from manifesting itself, even in the presence of a quantity of epinephrin more than sufficient to evoke good reactions, until the control of the remaining sympathetic fibers was removed.

It will be seen from the protocol that after section of the cord between the fifth and sixth cervical segments excellent pupil and nictitating membrane reactions were still obtained. The reactions were not noticeably less for equal periods of collection of blood in the pocket than before the section, although the interval after which they occurred was somewhat lengthened, corresponding to the slower blood flow. Subsequent division of the major and minor splanchnics in the abdomen greatly weakened the reactions and increased the time of collection necessary to elicit even a feeble response. Further section of fibers coming to the adrenals abolished the reactions even

with very long periods of collection. This was not due to total loss of sensitiveness of the reactive structures in the eye. For even at the end of the experiment a slight but definite response was still obtained on injection of 0.002 mg. of adrenalin.

As a first approximation towards defining the lower limit of the region of the cord concerned in the spontaneous liberation of epinephrin the following experiment was performed, both eye reactions and rabbit segment tests being employed.

Experiment 4. Condensed Protocol.—Cat; weight 1.72 kilos. Left superior cervical ganglion excised 8 days before the experiment.

10.00 a.m. 3.5 gm. of urethane.

11.20 a.m. Tracheal cannula inserted. Cervical and dorsal cord exposed for about a segment at each point. Long cava pocket made and arteries (renal, celiac, mesenteric, and abdominal aorta) tied.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
<i>p.m.</i>				
12.15	Pocket experiment.	1 min., 10 sec.	Good; in 8.8 sec.	Positive; 11 sec.
12.18	“ “	2 “	Very good; in 8.8 sec.	Very good; 8.8 sec.
12.22	Cut cord between fifth and sixth cervical vertebræ.			
12.32	Pocket experiment.	1 min., 45 sec.	Very good; in 10.8 sec.	Very good (a little later).
12.35	“ “	2 “	Very good; in 11.2 sec.	Very good (a little later).
12.40	Cut cord at fourth dorsal vertebra.			
12.49	Pocket experiment.	2 min.	None.	None.
12.55	“ “	3 “	“	“
	In the last two pocket observations the filling was slower than before.			
1.07	Pocket experiment with stimulation of cord between fifth and sixth dorsal vertebræ.	3 min.	Very good; in 20.2 sec.	Very good; 20.2 sec.

1.20 p.m. Put cannula in lower end of cava, making a short pocket, and collected two specimens of adrenal blood. First specimen, 1.6 gm. in 5 min.,

45 sec. (0.3 gm. per min.); second specimen, 3.7 gm. in 20 min. (0.2 gm. per min.). With the pocket still clipped off, blood was obtained from the abdominal aorta.

The left adrenal weighed 0.214 gm. and contained 0.12 mg. of epinephrin; the right adrenal weighed 0.238 gm. and contained 0.12 mg. of epinephrin.

The experiment shows that after transection of the cord between the fifth and sixth cervical vertebræ (through the fifth cervical segment, just below the fifth pair of nerve roots, as found at autopsy) the eye reactions were elicited by the adrenal blood, apparently in the same strength for a given time of closure of the cava pocket as before the section, although the interval between release of the pocket and the beginning of the pupil dilatation was somewhat lengthened, as would necessarily be the case owing to the lowered blood pressure and diminished speed of the blood. When the cord was now cut between the fourth and fifth dorsal vertebræ (between the third and fourth segments, as shown at autopsy) no eye reactions could be obtained even with longer periods of occlusion of the pocket than sufficed to cause excellent reactions just before. That the negative result was not due to diminished blood flow, but that the adrenals were still capable of secreting epinephrin actively, was proved by stimulating the cord electrically by needle electrodes inserted one above the fifth, and the other above the sixth dorsal spine while the adrenal blood was being collected in the pocket. Very good eye reactions followed the opening of the pocket, naturally after a longer interval than before the dorsal section, corresponding to the slower blood flow. The adrenal blood specimens now drawn off were so small that it was not quite certain whether some of the epinephrin in them might not have been liberated during the manipulations in inserting the cannula. Despite this, however, the concentration found even in the first specimen was somewhat less than 1:17,000,000, corresponding to an output of epinephrin per minute of 0.00002 mg.; that is, far below any concentration or output ever met with in the cat with intact adrenal innervation.

The experiments next to be considered, in which the animals were allowed to survive 2 or 3 days after the cord section³ before the epinephrin output was tested, so that any possible irritative discharge

³ All the operations were performed under ether anesthesia.

might be eliminated, yield clear evidence that transection of the dorsal cord at the level mentioned in the last experiment, or even a segment higher reduces the rate of liberation almost to zero. Transection one segment lower abolished the liberation entirely, or at least reduced it so much that no epinephrin could be detected in the adrenal blood by sensitive rabbit intestine and uterus segments. The upper limit of the portion of the cord related to the spontaneous liberation of epinephrin was also further defined by survival experiments and these may be taken first.

Experiment 5. Condensed Protocol.—Cat; weight 2.17 kilos. Cord transected at the level of the body of the last cervical vertebra 3 days before the experiment. Animal in fairly good condition. Anesthetized with ether for the insertion of the tracheal cannula; thereafter no more ether was required as the operative field was, of course, absolutely anesthetic because of the previous spinal cord section. Cava pocket made with ligation of all the usual arteries. Cannula inserted in lower end of pocket (short pocket) and the following samples of adrenal blood collected.

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	<i>gm.</i>		<i>gm.</i>
1	2.4	4 min., 50 sec.	0.5
2	2.2	7 " 30 "	0.3
3	2.1	9 " "	0.23
4	2.2	17 " 30 "	0.13

While the pocket was still clipped off blood was obtained from the abdominal aorta. While the pocket was being tied off some blood was left in it and the first adrenal specimen was therefore somewhat diluted. The autopsy showed that the cord had been divided between the last cervical and the first thoracic segments. Combined weight of adrenals 0.362 gm.

In survival experiments it is not so easy as in acute experiments to decide whether transections of the cervical cord leave the rate of liberation of epinephrin unaltered, or somewhat diminish it because the rate before and after the section cannot be compared on the same animal within a short interval of time. All that can be done is to determine whether the residual output after the cervical section is within the range established for animals under the same experimental conditions, but with intact central nervous system. In Experiment

5, as will be seen from the specimens of rabbit intestine and uterus tracings reproduced in Figs. 4 to 6, concentrations of epinephrin within the normal range² were found in the adrenal blood samples collected after section of the cervical cord between the last cervical and the first thoracic segments.

The adrenalin assays on the intestine segments gave a concentration, in the fourth adrenal specimen, of about 1:1,500,000 (Fig. 4, Observation 19; Fig. 5, Observation 27); and in the second adrenal specimen a concentration greater than 1:2,500,000 and less than 1:1,500,000 (Fig. 4, Observation 21; Fig. 5, Observations 29 and 27). Taking the concentration in the second specimen as 1:2,000,000, we get a liberation of epinephrin per minute of not quite 0.0002 mg. (0.0001 mg. per kilo of body weight). It may be considered certain that the output which the glands would have been capable of maintaining with a more nearly normal blood flow was at any rate not less than this. It might have been more, since as already mentioned, we rarely encounter concentrations of more than 1:1,000,000 in adrenal blood collected under our experimental conditions; and therefore the point might already have been passed at which the declining blood flow can be compensated by increased concentration of epinephrin.

The fact that it was not necessary to administer an anesthetic in order to collect the adrenal blood in this animal, since the cord section had rendered the operative field totally insensitive, has probably no bearing on the question whether the rate of output was somewhat diminished by the cervical section. For although some writers have assumed that anesthetics markedly increase the rate of liberation of epinephrin, there is no real proof of this. In any case, if the anesthetic exerts its effect through the higher parts of the central nervous system, the administration of an anesthetic could not have increased the output of epinephrin in this animal. In reference to the experiments (Nos. 1 to 4) in which the epinephrin output was determined immediately after section of the cervical cord, it might be asked, however, whether the anesthetic had not already abolished the activity of any portion of the brain or bulb which might be related to the epinephrin secretion. Division of the cervical cord would in that case cause no diminution in the output of epinephrin if made above the level of the spinal center. An assumption equally plausible, but at present equally devoid of experimental basis, is that anesthetics abolish or lessen an inhibition of the spinal center from a center in the brain. On this hypothesis, the output of epinephrin seen after cervical cord section would be considered as greater, not smaller, than the normal output with intact central nervous system.

The progressive increase in the concentration of epinephrin in successive adrenal blood samples associated with gradual diminution in the rate of blood flow is well brought out in the intestine tracings reproduced in Fig. 4, and still better in the uterus tracings in Fig. 6. This phenomenon and what underlies it—the stability in the rate of epinephrin discharge—is so characteristic when adrenal blood is collected with intact central nervous system, that its occurrence after spinal section lends support to the conclusion that the secretion of epinephrin when the connection of the cord with the brain has not been interrupted is also sustained largely, if not entirely, from the cord.

In the next experiment the eye reactions were studied in a cat 2 days after section of the cord just above the body of the seventh cervical vertebra.

Experiment 6. Condensed Protocol.—Cat; weight 2.825 kilos. Left superior cervical ganglion excised 1 week, and spinal cord divided 2 days before the experiment, just above the body of the last cervical vertebra.

10.00 a.m. Anesthetized with ether while the tracheal cannula was being inserted; thereafter no more ether was required as the operative field was necessarily absolutely insensitive because of the previous section of the cord.

10.05 a.m. The abdomen was opened and the cava pocket made, all the usual arteries being tied.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
<i>a.m.</i>				
10.28	Pocket experiment.	1 min.	Small; in 20 sec.	Small (shortly after pupil).
10.30	“ “	2 “	Good; in 15 sec.	Good; 15 sec.
10.45	Injected 0.5 cc. (1: 330,000) of adrenalin into jugular vein.		Very good; in 15 sec.	Very good; 15 sec.
10.50	Injected 0.25 cc. (1: 330,000) of adrenalin into jugular vein. The eye reactions after this injection were about the same or slightly less than in the observation at 10.30 a.m.		Good; in 17.2 sec.	Good; 17.2 sec.
10.55	Pocket experiment.	1 min.	Very slight; in 25 sec.	None.
10.58	“ “ The reactions were about the same as at 10.50 a.m.	2 “	Good; in 21 sec.	Good; 21 sec.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
<i>a.m.</i>				
11.02	Pocket experiment. The reactions were about the same as at 10.45 a.m.	3 min., 40 sec.	Very good; in 16.8 sec.	Very good; 16.8 sec.
11.10	Cut nerves to both semilunar ganglia in abdomen.			
11.20	Pocket experiment. Pocket not so well filled as before.	3 min.	None.	None.
11.25	Pocket experiment. The reaction was about the same as at 10.58 a.m.	5 "	Distinct; in 23.4 sec.	Distinct.
11.30	Both semilunar ganglia extirpated.			
11.37	Pocket experiment.	5 min.	None.	None.
11.45	" " Pocket filled very slowly.	8 "	Slight; in 41 sec.	"
11.55	Cut lumbar sympathetic chain just below diaphragm.			
12.00	Pocket experiment. Very poor flow.	25 min.	Slight; in 90 sec.	None.
<i>p.m.</i>				
12.50	Pocket experiment. Better flow.	10 "	Slight; in 60 sec.	"

The autopsy showed that the cord was divided through the seventh cervical segment, immediately below the origins of the seventh pair of nerves.

The fact that adrenal blood collected in the cava pocket caused good eye reactions was ascertained. It was shown by the injection of adrenalin solution that the amount of epinephrin secreted per minute was about 0.0004 mg. (0.00015 mg. per kilo of body weight per minute); this is a substantial output, although considerably less than the average, as estimated by eye reactions, in cats with intact central nervous system.⁴ Various nerves going to the adrenals were then divided and the effect in diminishing the eye reactions was noted. After division of the fibers coming to the semilunar ganglia, the eye reactions elicited by adrenal vein blood collected in the cava pocket were markedly diminished. A 3 minute collection gave no reactions, whereas before the nerve section a 1 minute collection caused a slight

⁴ Stewart and Rogoff, *J. Pharm. and Exp. Therap.*, 1916-17, ix, 479.

effect on the pupil, and a 2 minute collection good dilatation of the pupil and retraction of the nictitating membrane. After removal of both semilunar ganglia, which, of course, insured the section of any strands coming to the ganglia overlooked in the previous section, a 5 minute collection of adrenal blood caused no eye reactions, although previously a 2 minute collection gave good reactions. On release of a pocket occluded for 8 minutes, a small dilatation of the pupil was obtained.

In connection with the fact that slight eye reactions were still elicited with long periods of closure of the cava pocket even after extensive section of possible nerve paths to the adrenal glands, it must be pointed out that these nerve sections entail considerable manipulation of, and in the neighborhood of, the adrenals. With the slow blood flow toward the end of the experiment, epinephrin liberated by massage would take long to be completely washed out. It may be concluded that even the feeble reactions obtained after these nerve sections were not due entirely to genuinely secreted epinephrin. Survival experiments published elsewhere² have shown that the epinephrin output after section of the adrenal nerves is either abolished or reduced so much as to be incapable of detection by sensitive rabbit intestine and uterus segments. On the other hand, in acute experiments after the same nerve sections, although the output of epinephrin is greatly reduced, a content capable of detection is usually still found in adrenal blood.

It may, therefore, be confidently assumed that the whole output of epinephrin from the adrenals after section of the cervical cord near its lower limit is mediated through the same nerves which are concerned in the liberation with intact nervous system.

The last two survival experiments to be quoted were made for the purpose of defining more exactly the lower limit of the spinal region concerned in the epinephrin secretion.

Experiment 7. Condensed Protocol.—Cat; weight 1.59 kilos. Left superior cervical ganglion excised 6 days before the experiment and spinal cord transected between the fifth and sixth thoracic vertebræ 3 days before the experiment. Condition good. The autopsy showed cord section between the fifth and sixth thoracic segments.

2.00 p.m. Ether was given while the tracheal and jugular cannulas were

inserted. Thereafter no more ether was required as the operative field was, of course, totally insensitive owing to the cord section. A specimen of jugular blood was obtained. A long cava pocket was prepared; the renal arteries and abdominal aorta were tied.

2.45 p.m. Pocket experiment, 1 minute, 25 seconds. No eye reactions.

2.48 p.m. Pocket experiment, 3 minutes, 20 seconds. No eye reactions.

The blood flow was good; the pocket filled well.

3.00 p.m. Intestinal arteries tied. Cannula put in lower end of pocket (now made into a short pocket). Adrenal blood specimens collected as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	<i>gm.</i>		<i>gm.</i>
1	1.5	1 min., 25 sec.	1.0
2	3.2	4 " 30 "	0.7
3	3.5	10 "	0.35
4	2.2	10 "	0.22

Through a cannula in the jugular vein inserted under ether anesthesia the following injections were made: 0.5 cc. (1:500,000) of adrenalin; good pupil and nictitating reactions in 15 seconds. 0.2 cc. (1:500,000) of adrenalin; small nictitating reaction in 17 seconds; small pupil reaction in 25 seconds. While the pocket was still clipped off blood was obtained from the abdominal aorta.

Left adrenal weighed 0.206 gm. and contained 0.20 mg. of epinephrin; the right adrenal weighed 0.194 gm. and contained 0.18 mg. of epinephrin.

In this cat 3 days after transection of the cord between the fifth and sixth thoracic segments no eye reactions could be evoked by adrenal blood even with relatively long periods of collection in the cava pocket, periods which would certainly have given good reactions either with intact central nervous system or with the cord cut in the cervical region. The reactions were not lacking because the iris and nictitating membrane were incapable of responding to small quantities of epinephrin. For even at an advanced period in the experiment, after the withdrawal of several samples of adrenal blood through a cannula in the cava, good eye reactions were obtained on injection of 0.001 mg. of epinephrin, and quite detectable reactions on injection of 0.0004 mg. Tested with rabbit intestine and uterus segments, the adrenal blood gave a negative result (Fig. 7, Observation 27; Fig. 8, Observations 6 and 12), although a concentration of epinephrin of 1:60,000,000 (Fig. 8, Observation 37) could easily have

been detected by the intestine. There was evidence that the adrenal blood (third specimen) could not have contained even 1:100,000,000 epinephrin, corresponding to an output of at most 0.000003 mg. per minute, not one-hundredth of the output to be expected in a normal cat under the experimental conditions. It must be repeated that there was no evidence that any epinephrin was being discharged by the adrenals. Nothing could be more striking than the contrast between the concentration and output per minute of epinephrin in this cat and in those whose spinal cord was transected towards the lower level of the cervical region. For instance, the concentration in the second adrenal specimen in Experiment 5 was 1:2,000,000; and the calculated output per minute, 0.0002 mg. Even when the cord was divided (Experiment 8) two segments higher in the dorsal region, through the third thoracic segment, although a slight epinephrin liberation was detected by intestine segments, the output was enormously reduced (to 0.000006 mg. per minute).

Experiment 8. Condensed Protocol.—Cat; weight 3.65 kilos. Left superior cervical ganglion excised 10 days before the experiment. Spinal cord transected between the third and fourth dorsal vertebræ 3 days before the experiment. Condition excellent. Ether anesthesia was used throughout the experiment. Long cava pocket prepared in the usual manner, renal, celiac, and mesenteric arteries, and abdominal aorta being tied.

Time.		Duration of pocket.	Pupil dilatation.	Reaction of nictitating membrane.
<i>a.m.</i>				
11.00		1 min., 20 sec.	None.	None.
11.05		1 " 50 "	"	"
11.10		3 "	"	"
11.15		5 "	"	"
11.30	Injected 0.5 cc. (1:530,000) of adrenalin into jugular vein.		Excellent; in 10.6 sec.	Excellent; 10.6 sec.
11.35	Injected 0.2 cc. (1:530,000) of adrenalin into jugular vein.		Very good; in 14.8 sec.	Very good; 14.8 sec.
11.38	Injected 0.5 cc. (1:2,000,000) of adrenalin into jugular vein.		Small; in 22 sec.	None.
11.40	Injected 0.5 cc. (1:2,000,000) of adrenalin into jugular vein.		Small; in 22 sec.	"

11.55 a.m. Obtained indifferent blood from the other jugular.

12.00 m. Inserted cannula in cava (short pocket) and collected adrenal blood as follows:

No. of adrenal specimen.	Blood collected.	Time of collection.	Blood flow per min.
	<i>gm.</i>		<i>gm.</i>
1	3.6	2 min.	1.8
2	8.8	6 "	1.46
3	5.0	6 "	0.83
4	5.5	7 " 30 sec.	0.73
5	4.0	6 "	0.66

While the pocket was still clipped off a second specimen of indifferent blood (jugular) was obtained. The autopsy showed that the spinal section (between the third and fourth dorsal vertebræ) was through the origins of the third pair of thoracic nerves. The bloods were tested on rabbit segments. The right adrenal weighed 0.200 gm. and contained 0.18 mg. of epinephrin; the left adrenal weighed 0.208 gm. and contained 0.18 mg. of epinephrin.

The concentration of epinephrin in the third adrenal specimen (Fig. 9, Observation 30) was distinctly less than 1:135,000,000 (Observation 32), and very much less than 1:70,000,000 (Observation 28). The concentration in the fifth adrenal specimen was somewhat greater than 1:135,000,000. The eye reactions were negative, even when the cava pocket was closed for as much as 5 minutes, although the injection of 0.00025 mg. of epinephrin gave a definite pupil dilatation, and the injection of 0.0004 mg. a very good dilatation of the pupil and retraction of the nictitating membrane.

The results of the whole series of experiments are singularly consistent, particularly in view of the fact that there has been no selection of experiments. The eight experiments comprise all those performed, except one which has not been reported because the animal died before it was satisfactorily completed.⁵ The results indicate

⁵ In this animal the cord was divided between the second and third thoracic vertebræ, just below the origins of the second thoracic nerves, or nearly a segment higher than the highest dorsal section in the experiments reported. 3 days afterwards the eye reactions (the left superior cervical ganglion had been excised a week before the experiment) were found negative with collections in the cava pocket up to more than 3 minutes, although very good pupil and nictitating reactions were elicited by injection of 0.0015 mg. of epinephrin, and slight reactions by the injection of 0.0006 mg. The blood flow at the time of these observations was quite satisfactory, but the animal died be-

clearly that there exists in the cord between the last cervical segment and the fourth thoracic segment a mechanism which sustains the output of epinephrin from the adrenal glands after the cord is severed from the higher parts of the central nervous system. The experiments prove definitely that the center does not extend lower than the thoracic segment mentioned, and that at least an important part of it lies below the level of the last cervical segment. The possibility, however, is not excluded that the center may extend for some distance above the last cervical segment. It is of interest in connection with the currently accepted view of the development of the adrenal medulla, that the portion of the cord at which the sympathetic outflow begins should be identified as a center controlling the liberation of the only constituent of its secretion hitherto definitely recognized. If epinephrin in the quantities and concentrations in which it appears in the adrenal blood could be shown to fulfil an important office in maintaining the function of the sympathetic by activating certain of its elements, or by heightening or prolonging the effects resulting from its excitation, the location of an epinephrin center in the sympathetic region of the cord might perhaps acquire a new significance.⁶ It might

fore adequate adrenal blood samples could be drawn off from the cannula in the cava. The small quantity of adrenal blood obtained while the blood was flowing very slowly showed a good concentration of epinephrin as tested on the rabbit segments. Since the eye reactions cannot in general detect outputs of epinephrin easily detectable by the rabbit segments, this result, although no great stress can be laid upon it in the absence of better samples of adrenal blood, is quite consistent with the general conclusion deduced from the other experiments as to the position and limits of the portion of the cord concerned in epinephrin secretion from the adrenals.

⁶ It is difficult to demonstrate that the epinephrin spontaneously liberated from the adrenals has any effect upon the blood pressure unless its action is accumulated by collecting the adrenal blood in a cava pocket and then releasing it. The majority of recent observers have not seen any change in the blood pressure when the adrenal veins are carefully clipped. On the denervated eye, however, we have observed a phenomenon which indicates that even the small concentrations of epinephrin which can exist in the capillary blood when the adrenal blood is passing steadily into the circulation without being accumulated in the cava pocket can produce a demonstrable effect upon these extraordinarily sensitive objects. When the pupil has been dilated or the nictitating membrane retracted by release of adrenal blood collected in the cava pocket, the dilatation

then be permissible to speculate upon the possibility that the relative constancy of the epinephrin discharge, so puzzling on the hypothesis that it directly influences physiological events in virtue of the truly gross changes necessary to produce a detectable hyper- or hypoadrenalinemia, is associated with a more general and permanent action upon the sympathetic mechanisms, which does not entail the necessity of abrupt outbursts and remissions in the rate of liberation. To employ a simile which is doubtless excessively crude: if epinephrin is not the horse in the sympathetic machine, which must go now faster, now slower; nor even the whip which must sometimes be wielded vigorously and then be laid aside, is it not perhaps the lubricant which, whether the axle turns fast or slow, need not vary much in amount?

The possibility must, however, not be lost sight of, that epinephrin although the first definite constituent of the adrenal secretion to be discovered is not the only, nor the most important one which exists. It is difficult to conceive of a nervous control so complete as that which governs the output of epinephrin being developed in the case of a substance functionally unimportant. Yet, as we have shown in another place,² the output of epinephrin from the adrenals in cats is greatly and permanently reduced or abolished by section of the adrenal nerves without apparently interfering with the life or health of the animal. Section of the dorsal cord which, as has been shown above, produces a similar effect upon the output of epinephrin is also well known to be compatible with good health and long survival. Is there perhaps some as yet unknown substance of more importance than epinephrin which is normally given off from the adrenals under the influence of nerves, the secretion of which is eventually resumed after the nerves have been severed?

SUMMARY.

1. After section of the spinal cord in cats in the cervical region, as low as the last cervical segment, epinephrin continues to be liberated from the adrenal glands. This liberation has all the characters of the pupil disappears more slowly and the nictitating membrane comes forward more gradually when the pocket is left open than when it is clipped. Obviously, the steadily liberated epinephrin exerts an effect in prolonging the reactions once they have been elicited.

the normal secretion with intact central nervous system. It is sustained through the same nerve paths connecting the cord with the adrenals.

2. After section of the cord in the middorsal region the spontaneous liberation of epinephrin from the adrenals is abolished within the limits of detectability by the methods employed (denervated eye reactions of Meltzer, and rabbit intestine and uterus segments).⁷

3. The portion of the cord concerned in the liberation of epinephrin does not appear to extend much below the third thoracic segment.

4. In acute experiments on cats under urethane anesthesia no change in the rate of liberation of epinephrin, which could be detected by the tests employed, was observed when the cord was severed in the cervical region.

EXPLANATION OF PLATES.

PLATE 44.

FIG. 1. Intestine tracings. Blood from a cat. At 2 Ringer's solution was replaced by jugular blood, and this at 3 by the second adrenal blood specimen, collected with intact spinal cord. At 6 and 9 Ringer's solution was replaced by jugular blood, and this at 7 and 10 by the sixth and eighth adrenal blood specimens respectively, collected after section of the cord between the fourth and fifth cervical segments. All the bloods were diluted with four volumes of Ringer's solution. Reduced one-third.

FIG. 2. Intestine tracings. Blood of the same cat used for Fig. 1. At 35 Ringer's solution was replaced by arterial blood, and this at 36 by the second adrenal blood specimen, collected before section of the cord. At 43 Ringer's solution was replaced by arterial blood, and this at 44 by the third adrenal blood specimen, collected just after transection of the cord between the fourth and fifth cervical segments. All the bloods were diluted with three volumes of Ringer's solution. At 37 Ringer's solution was replaced by arterial blood diluted with three volumes of Ringer's solution, and this at 38 by arterial blood to which adrenalin had been added to make up a concentration of 1:13,000,000, the adrenalin blood being then diluted with three volumes of Ringer's solution. Reduced one-third.

⁷ We have since found that semisection of the cord (between the fourth and fifth dorsal segments in one cat, between the third and fourth segments in another) abolished the liberation of epinephrin from the adrenal of the same side without affecting the liberation from the other adrenal.

PLATE 45.

FIG. 3. Intestine and uterus tracings. Blood of the same cat used for Figs. 1 and 2. At 19 Ringer's solution was replaced by jugular blood, and this at 20 by the eighth adrenal blood sample, collected after section of the cord between the fourth and fifth cervical segments. Both bloods were diluted with eight volumes of Ringer's solution. At 21 and 23 Ringer's solution was replaced by adrenalin in jugular blood, made up to 1 : 1,600,000 and 1 : 800,000, respectively, and then diluted with eight volumes of Ringer's solution. 4, 5, and 6 are uterus tracings. At 4 Ringer's solution was replaced by the eighth adrenal blood specimen, at 5 by the second, and at 6 by indifferent (arterial) blood; all were diluted with seven volumes of Ringer's solution. Reduced one-third.

FIG. 4. Intestine tracings. Adrenal blood from a cat after section of the cord between the last cervical and first thoracic segments. At 18, 20, and 22 Ringer's solution was replaced by indifferent (arterial) blood, and this at 19, 21, and 23 by the fourth, second, and third adrenal specimens, respectively. All the bloods were diluted with two volumes of Ringer's solution. Reduced one-half.

FIG. 5. Intestine tracings showing some of the adrenalin assays for the adrenal bloods used for Fig. 4. At 26 and 28 Ringer's solution was replaced by indifferent (arterial) blood, diluted with two volumes of Ringer's solution and this at 27 and 29 by adrenalin in arterial blood, made up to 1 : 1,500,000 and 1 : 2,500,000, respectively, and then diluted with two volumes of Ringer's solution. Reduced one-half.

PLATE 46.

FIG. 6. Uterus tracings. Blood of the same cat used for Figs. 4 and 5. At 36 Ringer's solution was replaced by the third adrenal specimen; at 37, by the second, both diluted with eight volumes of Ringer's solution. The third specimen has a stronger effect than the second, corresponding to the slower blood flow during its collection. But as the increase of tone produced by the second specimen even was nearly maximal, greater dilution was necessary to show the difference clearly. At 38 Ringer's solution was replaced by the second adrenal specimen, and at 39 by the third, both diluted with twelve volumes of Ringer's solution. The difference is now evident. At 40 Ringer's solution was replaced by the fourth, and at 41 by the third adrenal specimen, both diluted with sixteen volumes of Ringer's solution. The increase of tone produced by the third specimen was now so nearly maximal for the condition of the segment at the time that greater dilution was resorted to, to bring out the difference, clearly seen in Observations 43 and 44, where Ringer's solution was replaced by the third and fourth specimens respectively, both diluted with twenty-four volumes of Ringer's solution. At 42 Ringer's solution was replaced by indifferent (arterial) blood, diluted with twenty-four volumes of Ringer's solution. Reduced one-half.

FIG. 7. Intestine tracings. Adrenal blood from a cat after section of the cord between the fifth and sixth dorsal segments. At 25 Ringer's solution was replaced by indifferent (arterial) blood, and this at 27 by the third adrenal specimen, both bloods being undiluted. The magnification is high, nearly twice as great as in Fig. 8, in order to afford the best chance for an inhibitory effect to be seen on the tracing. The writing point went somewhat above the drum after the addition of the adrenal blood and no inhibition was produced. To save space, the tracing has been cut horizontally. To reconstruct it, the right-hand portion may be imagined to be pushed up to the top of the figure and then to the left till it is in line with the left-hand portion.

PLATE 47.

FIG. 8. Intestine tracings. Blood from the same cat as in Fig. 7, but with a smaller magnification. At 5 and 11 Ringer's solution was replaced by arterial blood, and this at 6 and 12 by the fourth and the third adrenal specimens, respectively, the bloods being diluted with four volumes of Ringer's solution. At 34 and 36 Ringer's solution was replaced by arterial blood (undiluted), and this at 35 and 37 by adrenalin in arterial blood (1:40,000,000 and 1:60,000,000 respectively). The adrenalin blood was undiluted. Reduced one-third.

FIG. 9. Intestine tracings. Adrenal blood from a cat after section of the cord through the third thoracic segment. At 29 Ringer's solution was replaced by jugular blood, and this at 30 by the third adrenal blood specimen. At 27 and 31 Ringer's solution was replaced by jugular blood, and this at 28 and 32 by jugular blood to which adrenalin had been added to make up a concentration of 1:70,000,000 and 1:135,000,000, respectively. All the bloods were undiluted. Reduced one-third.

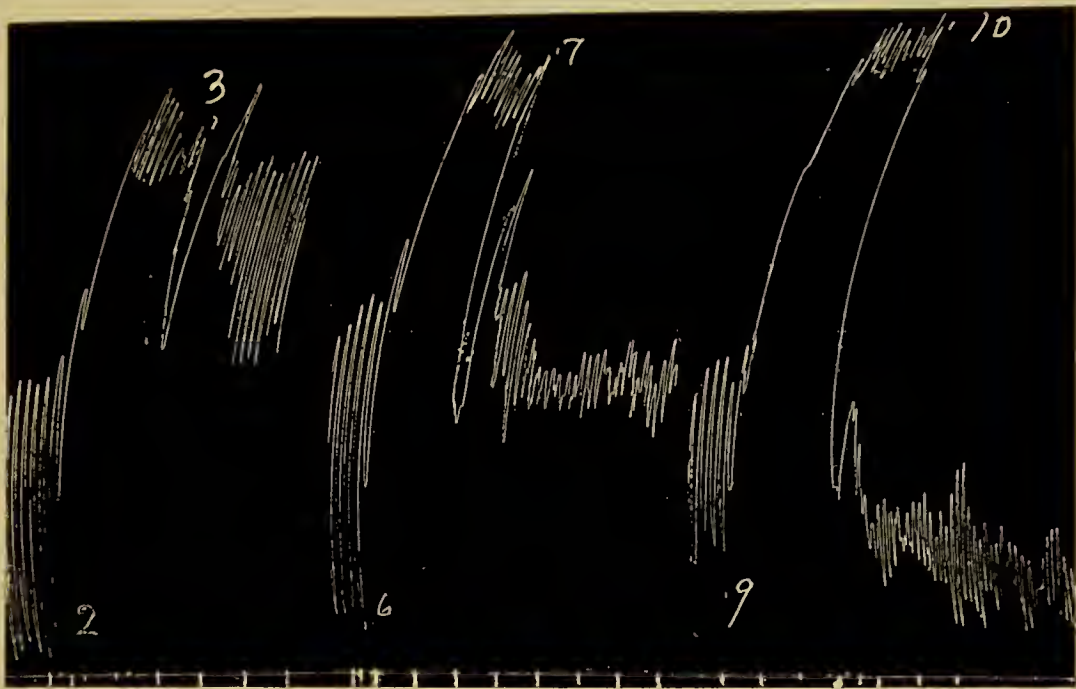


FIG. 1.

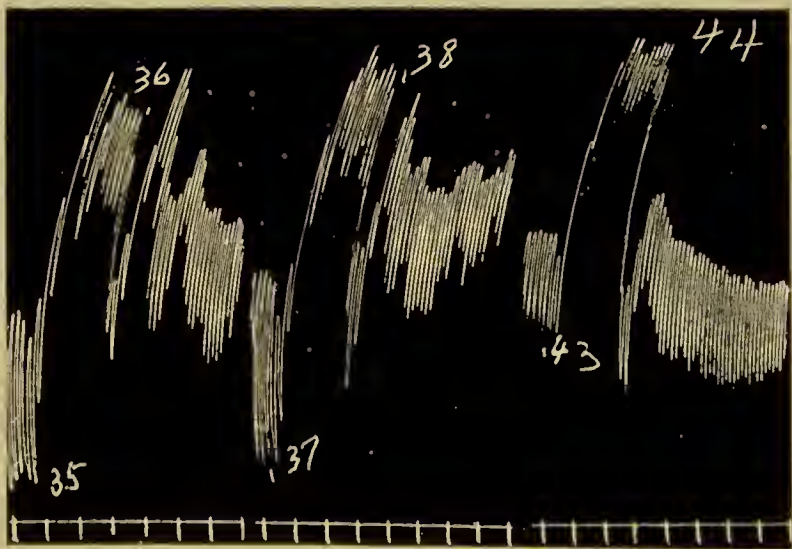


FIG. 2.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

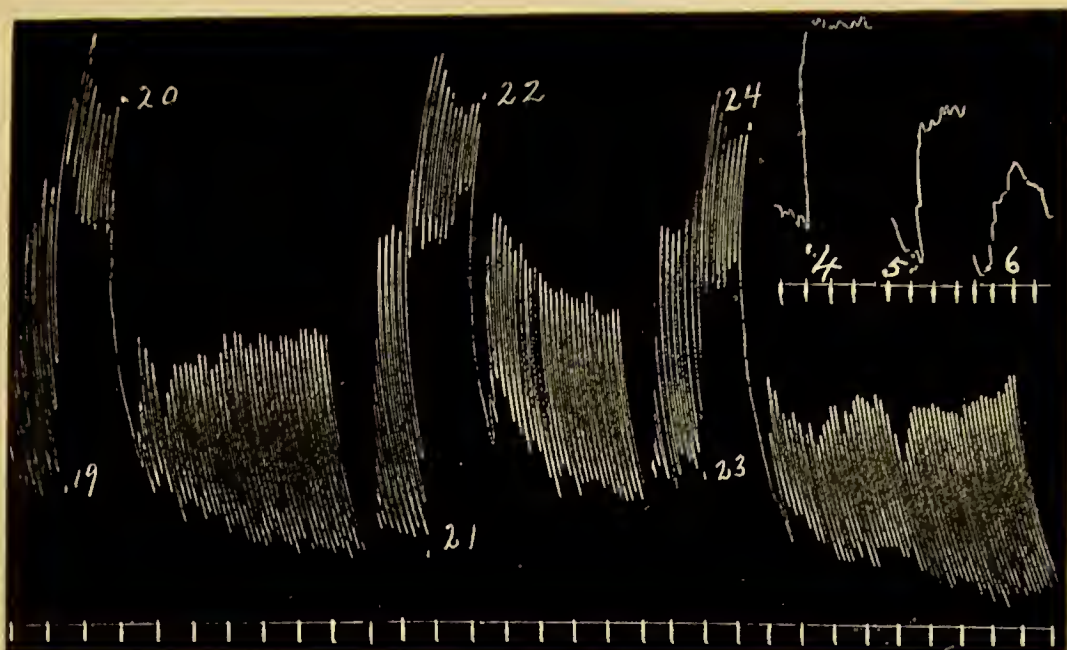


FIG. 3.

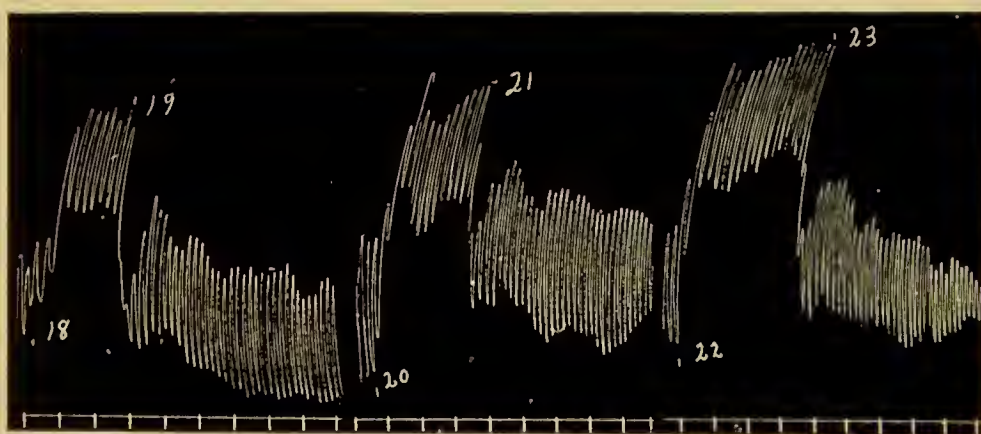


FIG. 4.

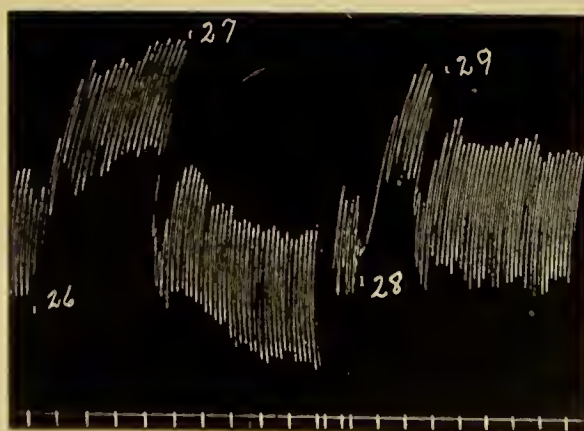


FIG. 5.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

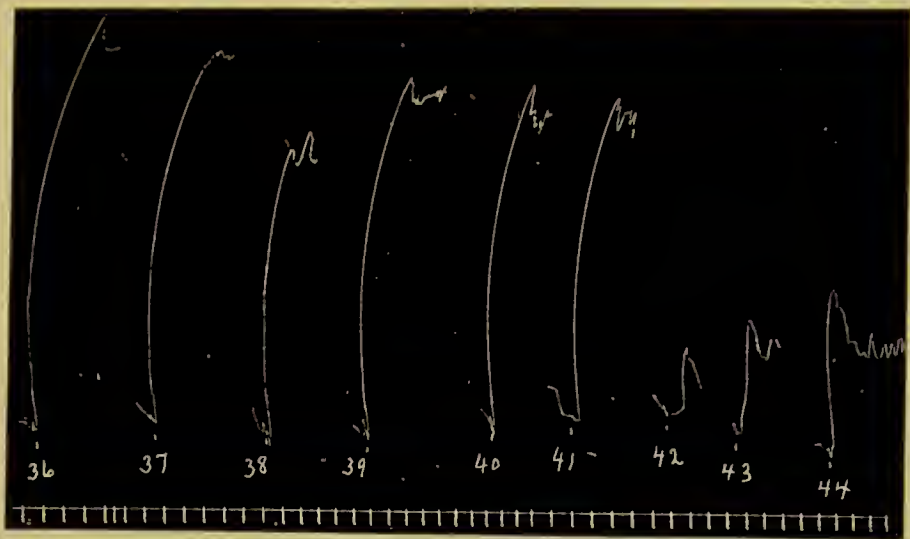


FIG. 6.

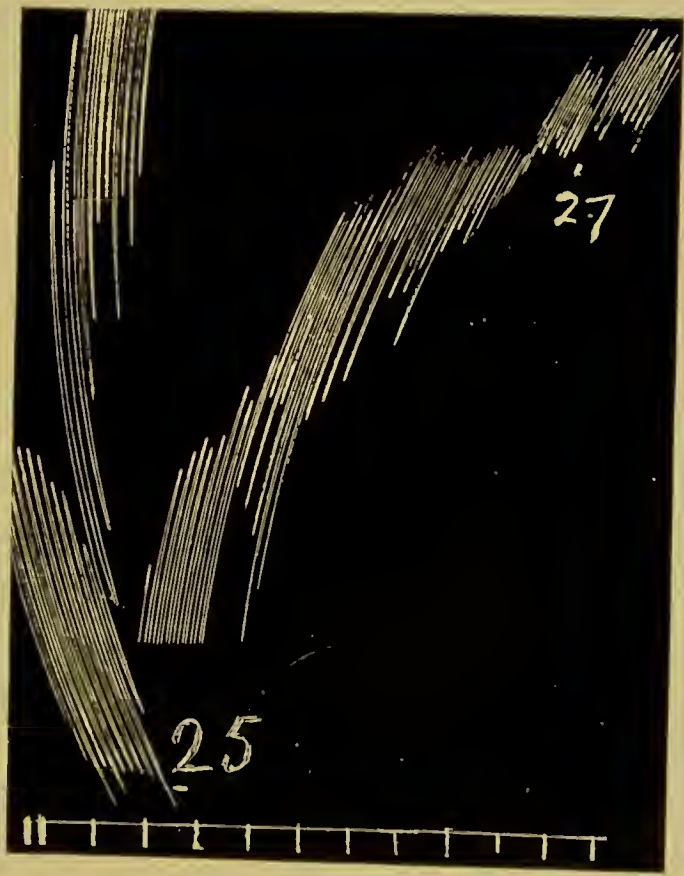


FIG. 7.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

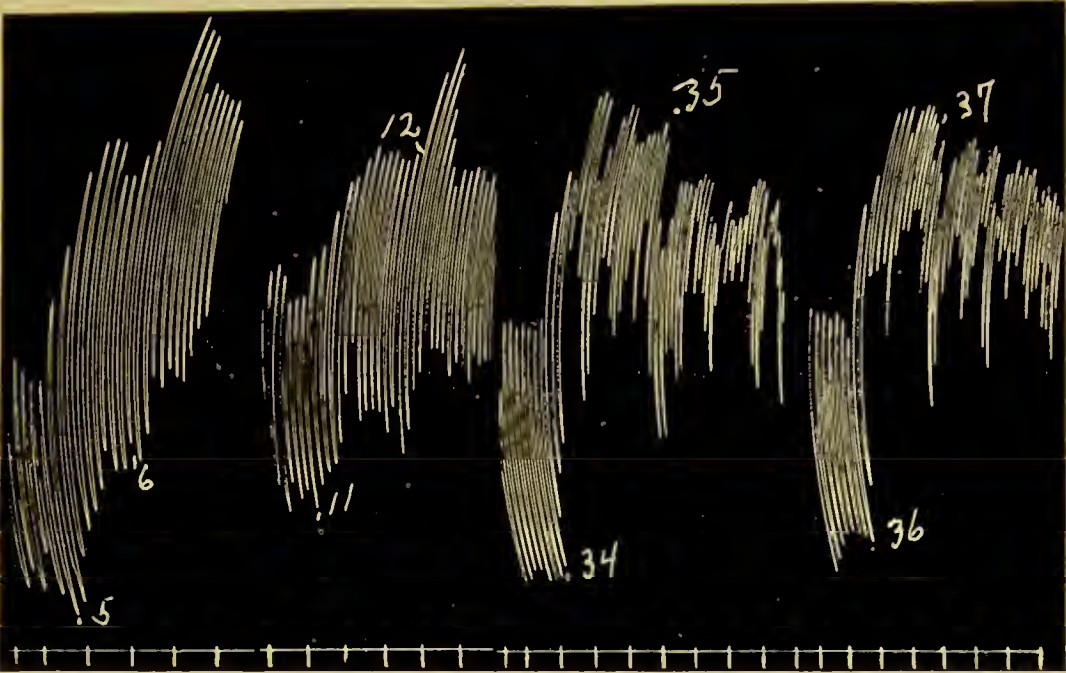


FIG. 8.

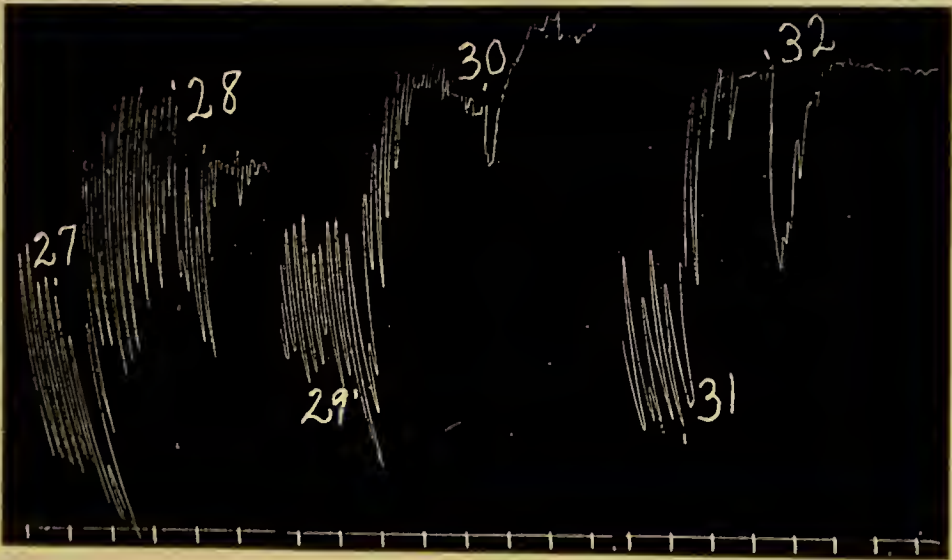


FIG. 9.

(Stewart and Rogoff: Spontaneous liberation of epinephrin.)

THE ALLEGED RELATION OF THE EPINEPHRIN SECRETION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPERGLYCEMIAS

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The view has been expressed by various writers that some, perhaps most, of the forms of experimental hyperglycemia (asphyxia (1), piqûre (2), etc.) are dependent primarily upon excitation of the adrenal glands to increased secretion of epinephrin, which then causes an accelerated mobilization of sugar in the same way as adrenalin does when artificially introduced. It is against this idea that subcutaneous injection of adrenalin is more effective in producing hyperglycemia and glycosuria than intravenous injection. However, it has been cited as evidence that the adrenal intervenes as an essential factor in the hyperglycemias in question, that a given experimental hyperglycemia which can be shown to occur while the adrenals are intact cannot be obtained after their removal. Further, some observers have stated that even the normal sugar content of the blood is not maintained after adrenalectomy, hypoglycemia being present; that glycogen cannot be normally stored by the liver, and so on.

The experimental basis for these conclusions seems to us to be quite unsatisfactory. Observations in which the production of hyperglycemia by a given procedure before removal of the adrenals are compared with similar observations after removal of the glands, suffer from two serious defects. In the first place, the animal has been deprived of organs essential to life. The period of survival is very brief in the absence of accessory glands, and when an animal is going to die within twenty-four hours it is surely a matter of difficulty and risk to fix the point up to which such a function as the regulation of the sugar content of the blood may still be regarded as normal. Secondly, the animal has been subjected to a major operation; it has been anesthetized for a time; possibly it has been prepared for the operation by a

period of fasting; certainly it does not eat after the operation. All these things cannot fail to complicate greatly any observations upon changes in the blood sugar dependent upon an increase or diminution of the rate of mobilization of the liver glycogen.

We have put the question of the relation of epinephrin to some of the forms of experimental hyperglycemia to the test by a method which eliminates all these disturbing factors. The epinephrin liberation was abolished, within the limits of sensitiveness of the methods used for its detection, or reduced to an insignificant fraction of its normal amount by dividing in cats the nerve supply of one adrenal and excising the other gland. After recovery from the operation the sugar was estimated from time to time in samples of blood obtained under the various conditions which it was desired to study, these being compared with normal samples previously collected.

TECHNIQUE

The sugar was estimated by the method of Lewis and Benedict (with Pearce's modification). Blood was collected from a vein, usually the femoral, by puncture with a hypodermic needle attached by a rubber tube to a 2 cc. pipette, in the point of which were a few crystals of potassium oxalate. The skin over the vein was shaved, usually the day before the blood collection, so that the animal might have recovered from the disturbance due to the shaving. Generally both legs were shaved at the same time so that blood could be obtained from either vein. Occasionally the jugular, and once or twice the external saphenous were employed. The needle was pushed directly through the skin into the vein. After the blood samples desired had been collected, a little tincture of iodine was placed on the skin over the site of the puncture, and a considerable interval, usually about a week, was allowed to elapse before any further samples of blood were taken. This refers to the observations in which such procedures as asphyxia and anesthetization had been resorted to. The blood was drawn up into the pipette a little above the mark, the pipette disconnected from the needle and the blood after being allowed to flow back to the mark, immediately discharged into a large test-tube containing 8 cc. of distilled water. The test-tube was then shaken. After hemolysis had occurred 15 cc. of saturated solution of picric acid was added and the contents filtered, after being well shaken. Duplicate quantities (7 cc.) of the filtrate were measured into test-tubes graduated in 0.1 cc. from 1 to 20 cc. Two cubic centimeters of saturated solution of picric acid and 1 cc. of 10 per cent solution of anhydrous sodium carbonate were added to each test-tube. After removal from the autoclave, any loss of fluid was replaced up to 10 cc. After filtration the color was compared with the picramic acid standard recommended by Lewis and Benedict, and also with a solution of dextrose of known percentage which was carried through the same process as the blood. Before deciding to use Pearce's modification, we compared it with the original method and found a sufficiently close agreement, as illustrated by the

following figures obtained with different quantities of a solution of dextrose. By the polarimeter it was estimated that 100 grams of the dextrose used corresponded to 93.6 grams dextrose. From this a solution was made of such a strength that 1 cc. would contain 0.936 mgm. dextrose.

DEXTROSE SOLUTION	LEWIS AND BENEDICT METHOD	AUTOCCLAVE MODIFICATION
cc.	mgm.	mgm.
1.0	0.881	0.890
1.0	0.910	0.940
2.0	2.060	2.012
0.5	0.418	0.411
0.5	0.461	0.427
0.75	0.748	0.741

In this paper are reported observations upon two forms of experimental hyperglycemia, that produced by asphyxia and that produced by ether anesthesia. The routine was to obtain a preliminary specimen of "normal" blood and then to subject the animal to a period of asphyxia or anesthesia. Asphyxia was maintained by placing over the nose and mouth of the animal a metal cone covered with a towel. The effect of the asphyxia was controlled throughout the whole period by palpating the heart through the chest wall. When distinct slowing of the heart had been produced the animal was allowed to breathe freely for a few seconds and the asphyxia was then repeated. The object was to produce and maintain with the necessary intervals of free breathing, a distinct asphyxial condition for ten to twenty minutes. The asphyxia was never pushed so far as to endanger life. As regards the anesthesia effect, an ordinary surgical anesthesia was maintained for fifteen to twenty minutes. In a few observations the etherization was less complete, stopping short of disappearance of the corneal reflex. In a number of the experiments when the animal happened to be especially quiet during the collection of the preliminary specimen, it was immediately afterwards subjected to frightening by a dog for twenty minutes to an hour, and another blood specimen then collected. Generally the animal was free in a small cage during the frightening, but in a few observations it was tied on a holder. In no case was it possible for the dog to inflict any physical injury on the cat. In some cases the sugar content of blood specimens, obtained after varying periods during which the animal remained tied down, was compared, although no systematic observations on the so-called "Fesselungs" hyperglycemia were made. The frightening experiments were followed by an asphyxia observation, in order to determine whether the animal was capable of showing a decided hyperglycemia. In this way it was supposed that a negative result due to poverty of the glycogen store of the liver could be taken account of. In a few of the preliminary observations single blood specimens were obtained, either "normal" or during asphyxia or anesthesia. Although the hyperglycemia associated with these conditions can be demonstrated without difficulty by such isolated observations made on different days on the same or on different animals, it is far better to compare a succession of samples taken on the same occasion from one and the same animal.

Control observations were made on normal cats and also on cats on which laparotomy had been performed, with a certain amount of manipulation of the abdominal viscera, in order to imitate the surgical procedure in the adrenal operations, except that the adrenals and their nerve supply were left intact. It was not necessary to estimate the epinephrin output in the control animals, as we have previously shown (3) that epinephrin is invariably present in the adrenal vein blood of normal cats when tested under our experimental conditions, and that the amount liberated per minute in different individuals varies within rather narrow limits. All the animals, those in which the adrenal operation had been performed as well as the controls, were kept on the same diet and housed together (in the open air for a large part of the time). Except when purposely restricted, the diet was such as to favor the accumulation of glycogen in the liver (rice with milk, pig's liver, with fish occasionally). In the observations before July 9, no rice was given. Glycogen in plenty was demonstrated in the liver in animals which were giving off no detectable epinephrin, (e.g., 4.75 and 4.26 per cent. in two operated cats; 1.95, 2.55 and 4.13 per cent. in three control cats).

EFFECT OF THE OPERATION ON THE OUTPUT OF EPINEPHRIN

Our previous experiments (3) showed to what insignificant proportions the liberation of epinephrin (as determined by the denervated eye reactions without drawing blood, or on rabbit intestine and uterus segments with shed blood) is reduced by the operation practiced, even when it is not completely abolished. The values for the residual liberation in the seven cats used for survival observations in that investigation are displayed in table 1. In five of the cats the output of epinephrin was only $\frac{1}{25}$ to $\frac{1}{170}$ of the average output for normal cats. In two of the seven animals no epinephrin whatever could be detected, although in one of them (cat 52) the intestine and uterus segments were so sensitive that $\frac{1}{1000}$ of the average normal output per kilogram per minute could have been estimated. In the other (cat 46) $\frac{1}{550}$ of the normal output could have been detected by the segments and $\frac{1}{600}$ by the eye reactions, which in this case happened also to be extraordinarily sensitive.

It is out of the question to assume that in this experiment epinephrin was present in the adrenal vein blood in a concentration just below the threshold of detectability for *both* rabbit segment and eye reactions. It is therefore as certain as anything can be which has not been actually demonstrated that if any epinephrin whatever was being given off in this animal, it represented much less than $\frac{1}{600}$ of the normal output.

We might then have assumed with confidence that the cats operated upon in a similar way for the experiments on hyperglycemia would be practically incapable of liberating epinephrin from the adrenals, either under normal circumstances or in conditions which have been supposed to cause increased liberation through the adrenal nerves. Never-

TABLE 1

NUMBER OF CAT	WEIGHT	EPINEPHRIN OUTPUT		FRACTION OF NORMAL LIBERATION*		DAYS AFTER OPERATION	EYE REACTIONS
		Per minute	Per kilogram per minute	Per animal	Per kilogram of animal		
	kgm.	mgm.	mgm.				
35	2.0	0.0000035	0.0000015	$\frac{1}{190}$	$\frac{1}{170}$	8	Very faint, if any
34	2.75	0.00003	0.00001	$\frac{1}{20}$	$\frac{1}{25}$	16	Negative
46	2.155	0.000001	0.00000045	$\frac{1}{650}$	$\frac{1}{550}^\dagger$	15	Negative; liberation of 0.000001 mgm. epinephrin per kilogram per minute (or $\frac{1}{600}$ of normal) could have been detected
52	3.87	0.0000009	0.00000025	$\frac{1}{700}$	$\frac{1}{1000}^\dagger$	20	Negative
31	2.63	0.00001	0.00004	$\frac{1}{65}$	$\frac{1}{60}$	105	Negative
32	4.14	0.00002	0.000005	$\frac{1}{30}$	$\frac{1}{50}$	105	Positive but slight
33	3.635	0.00002	0.0000055	$\frac{1}{30}$	$\frac{1}{45}$	106	Positive

* Since one of our objects in the previous investigation was to determine whether the whole secretion of epinephrin is dependent upon the integrity of the nerves, the results were expressed not only in fractions of a milligram per minute per animal, but also as fractions of a milligram per minute per kilogram of animal on the supposition that the animal still had two adrenals secreting at the same rate as the remaining gland. This showed when compared with the normal output of cats with both adrenals intact the extent to which the output of the remaining adrenal had been reduced by the operation. The residual liberation from the one adrenal expressed as a fraction of the normal liberation by one adrenal is obtained from the table by halving the denominators of the fractions in the fifth and sixth columns.

† There was no evidence that any epinephrin was being given off in these animals.

theless, in each animal the epinephrin output was determined at the end of the series of observations by the methods and under the experimental conditions previously employed for the normal cats. The results of the epinephrin estimations on the seven cats used for the blood sugar experiments after interference with the epinephrin output

are given in table 2. The adrenalin solution employed for the blood assay was always itself freshly assayed by the method of Folin, Cannon and Denis.

TABLE 2

NUMBER OF CAT	WEIGHT	EPINEPHRIN OUTPUT		FRACTION OF NORMAL LIBERATION		DAYS AFTER OPERATION	EYE REACTIONS
		Per minute	Per kilogram per minute	Per animal	Per kilogram of animal		
	kgm.	mgm.	mgm.				
90	1.5	0.0000035	0.000002	$\frac{1}{200}$	$\frac{1}{120}$ *	65	Negative
108	2.2	0.000005	0.000002	$\frac{1}{130}$	$\frac{1}{120}$ *	35	Negative. Could not have been 0.000005 mgm. per kg. per minute, or $\frac{1}{120}$ normal as determined by eye reactions
109	3.22	0.00001	0.000003	$\frac{1}{80}$	$\frac{1}{80}$	39	Negative. Could not have been $\frac{1}{80}$ of normal output per kg. per minute
107	1.27	0.00013	0.00001	$\frac{1}{50}$	$\frac{1}{25}$	34	Positive
91	2.06	0.0001	0.00005	$\frac{1}{6}$	$\frac{1}{5}$	61	Positive ($\frac{1}{5}$ of normal output per kg. per minute)
92	1.93	0.00002	0.00001	$\frac{1}{80}$	$\frac{1}{60}$ *	61	Negative
121†	1.7	0.000015	0.00001	$\frac{1}{40}$	$\frac{1}{25}$	24	Negative. The output could not have been more than $\frac{1}{30}$ of normal for the whole animal, or $\frac{1}{20}$ of normal per kg. of animal

* There was no evidence that any epinephrin was being given off.

† Left adrenal excised; nerves of right adrenal cut.

It will be seen that the results are precisely the same in these seven cats as in the seven reported in table 1. In two of them no epinephrin whatever was detected in the adrenal vein blood by the segment tests. No attempt was made to fix the minimum concentration of epinephrin which the segments could detect but it was shown that good reactions were still given with concentrations which with the observed blood flows through the adrenals would have corresponded to an output per kilogram of body weight per minute one hundred and twenty times less than the average for normal cats. There is no doubt that the quantity which could possibly have been present was still smaller. In cat 108 the eye tests gave as the possible maximum output the same fraction, $\frac{1}{120}$ of the normal, as the intestine tests. Again, it is extremely unlikely that had the reactions been a little more sensitive they would have detected epinephrin. It is much more probable that just as in the series shown in table 1, the fortunate coincidence

of specially sensitive test objects and blood specimens from animals in which the secretory nerves had been severed with unusual completeness would have enabled us to drive down the limit of the possible epinephrin output far beyond that actually obtained. The question, however, is of no consequence for our purpose. For an animal which cannot be liberating $\frac{1}{200}$ of the normal amount of epinephrin, owing to section of secretory nerve fibers, is certainly no more capable of responding to stimulation of any remaining fibers by an outburst, bringing the output far above the normal, than if it had been shown that the rate of liberation had been reduced to $\frac{1}{1000}$ or $\frac{1}{10000}$ of the normal. It must always be remembered that no evidence was obtained in these animals that any epinephrin was being given off. It is obvious that in connection with the problem whether an experimental hyperglycemia depends upon increased epinephrin secretion, experiments in which no epinephrin has been detected with sensitive test objects are more important than those in which a small residual liberation is still present. In a third cat of this series (cat 92) the result of the epinephrin assay was also negative. But here there were only the eye reactions to go by, not enough blood having been obtained for satisfactory segment tests. Still, the eye reactions were quite sensitive and showed that the output per kilogram per minute could not have been $\frac{1}{60}$ of the normal average.¹

In one animal of the series (cat 91) a substantial fraction of the normal average output of epinephrin was found, $\frac{1}{3}$ or $\frac{1}{6}$ by the intestine segments and $\frac{1}{8}$ by the eye tests. There was no question that a marked diminution in the output per minute had been effected by the operation in this animal. For the concentration of epinephrin in the adrenal vein blood was far less than is ever seen in a normal cat under our experimental conditions, for the corresponding rates of adrenal blood flow. As the epinephrin assay was made sixty-one days after the nerve section, the possibility of some regeneration of the secretory fibers might be considered but we have no evidence as to this. If any regeneration had occurred in this time, the output of epinephrin determined at the end of the period would be greater than it was after section of the nerves. However, since this animal yielded precisely the same results as the others in the blood sugar observations, the question is of no significance for our present purpose.

To sum up, if tables 1 and 2 are compared it will be seen that the results of the operation, as practiced by us, upon the residual epinephrin secretion are of the same general character for the two series. In each group of seven cats two gave no evidence with either test of any epinephrin output whatever. In each group one cat showed a somewhat substantial residual liberation, although only a mere fraction of the

¹ Generally the eye reactions, as stated in a previous paper (3), will not detect such small outputs of epinephrin as the segment tests with shed blood. This depends, however, not only upon the threshold concentration and quantity of epinephrin which yield a just detectable reaction, but also upon the length of time during which it is feasible to continue the collection of the adrenal vein blood in the cava pocket, and this in turn depends upon the rate of blood flow through the gland.

normal. The remaining cats in each group were proved to be giving off a very small amount of epinephrin ($\frac{1}{40}$, $\frac{1}{50}$ down to $\frac{1}{200}$ of the normal), except cat 92 in the second group (table 2), which by the only tests applied, the eye reactions, yielded a negative result. If we reflect that to ensure the severing of the secretory innervation of the adrenal a far larger number of fibers which have nothing to do with the epinephrin secretion must be cut, it will readily be seen that the completeness with which the fibers in question are divided may vary in the different operations. Let this be as it may, the tables demonstrate conclusively that to all intents and purposes the epinephrin secretion by the adrenals may be considered as non-existent in cats after this operation and that any effect produced upon the blood sugar content by given conditions cannot be mediated through the nervous mechanism which normally governs the liberation of epinephrin from these glands.

One other remark may be made before passing from the consideration of these tables. It will be noticed that in table 2, the fraction of the normal output represented by the residual liberation expressed per kilogram of body weight is in general greater than the fraction expressed for the whole animal, while the opposite is the case in table 1. The reason for this, or at least the main reason, is a purely artificial one, namely that in table 2 the body weight of most of the cats is less than the body weight of the majority of those in table 1. This is partly a matter of accident but partly due to the fact that the cats in table 1, after the loss of weight which always occurs in the first weeks after the operation, remaining undisturbed by further interference, rapidly regained their original weight and in several instances became considerably heavier than before the operation. The animals in table 2 were used for the blood-sugar observations, samples of blood being repeatedly taken from them; they were subjected to periods of asphyxia or anesthesia, and naturally most of them lost some weight.

THE NORMAL BLOOD-SUGAR CONTENT IN THE CAT

It has already been stated that in determining whether an increase in the sugar content of the blood was caused by the conditions investigated, comparison was not made with an average "normal" content deduced from observations made at other times on the same or on other animals, but successive samples collected at the time of each experiment were compared. In testing out the technique, however, a number of normal sugar estimations were first made. Some of these are given in table 3, with the duplicate estimations; in the rest of the paper only the average of the duplicate observations is given. For convenience, estimations on two dogs are included in the table.

TABLE 3

NUMBER OF ANIMAL	PERCENTAGE OF DEXTROSE IN BLOOD			REMARKS
	Duplicates		Average	
1	0.106	0.115	0.11	Excited during collection
2	0.081	0.083	0.082	Considerably excited
3	0.108	0.108	0.108	Considerably excited (lost 10 cc. blood)
4	0.117	0.116	0.116	Struggled and cried
5	0.107	0.111	0.109	Very quiet
7	0.103	0.103	0.103	Excited
11	0.081	0.081	0.081	Quiet
19*	0.094	0.097	0.096	Much excited; blood dark
15†	0.113	0.114	0.114	Very quiet
16†	0.105	0.104	0.104	Apprehensive

* This cat was given no liver for two days before the blood samples were obtained, and no food at all for twenty-four hours, two days before the blood experiment. All the others were on the liver diet with milk daily, but no rice.

† Dogs: no. 15, a large female hound; no. 16, a small female fox terrier

Scott (4) has published numerous blood-sugar estimations on cats. He worked with large quantities of blood obtained by decapitation, precipitating the proteins by a special method and estimating the sugar by a method described by Munsen and Walker (5). Our "normal" results for cats agree fairly well with those given by him but are on the whole somewhat higher. We have not employed dogs for the adrenal operations because the nerve paths whose section abolishes or greatly lessens the epinephrin output are better known in the cat than in the dog.

Experiments on cats in which the epinephrin output was interfered with

Cat 90. Condensed protocol. Weight, 1.82 kgm. Right adrenal excised and nerves of left adrenal cut on May 9.

	BODY-WEIGHT		PERCENTAGE OF BLOOD-SUGAR
	<i>kgm.</i>		
May 22.....	1.625	Moderate asphyxia, 10 minutes	0.17
June 11.....	1.605	"Normal" blood. Restless	0.09
June 30.....	1.495	After being tied on board 3 minutes	0.129
June 28.....	1.665	After being tied on board 15 minutes	0.128
		Excised left superior cervical ganglion	

	WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
		Preliminary specimen	Ether	Asphyxia
	<i>kgm.</i>			
June 21.....	1.515	0.20*	0.233	
June 27.....	1.67	0.087	0.151	
July 2.....	1.78	0.07		0.168
July 7.....	1.635	0.089		0.164

* During collection of this specimen the blood flowed very slowly and was very dark.

July 13. Weight, 1.5 kgm.

10.00 a.m. 3 grams urethane by stomach tube.

10.30 a.m. Prepared cava pocket, tying all arteries, i.e., renal, coeliac and mesenteric arteries and abdominal aorta.

11.20 a.m. Pocket experiment, 2 minutes occlusion; no eye reactions.

11.30 a.m. Pocket experiment, 4 minutes occlusion: no eye reactions.

11.35 a.m. Pocket experiment, 6 minutes occlusion: no eye reactions.

11.38 a.m. 0.5 cc., 1:1,000,000 adrenalin injected; very slight pupil, no nictitating reaction.

11.45 a.m. Repeated last observation with same result.

Then collected the following specimens of adrenal blood:

First specimen, 1.5 grams in $1\frac{1}{2}$ minutes, blood flow 1 gram per minute.

Second specimen, 4.5 grams in 5 minutes, blood flow 0.9 gram per minute.

Third specimen, 5.8 grams in 7 minutes, blood flow 0.83 gram per minute.

Right adrenal weighed 0.206 gram, and contained 0.18 mgm. epinephrin.

Left adrenal weighed 0.186 gram, and contained 0.17 mgm. epinephrin.

Some of the tracings illustrating the epinephrin assay are reproduced in figures 1 to 3. Figure 1 shows that the second adrenal specimen diluted with three volumes of Ringer's solution caused no inhibition of the intestine (observation 21), and that the undiluted blood could not have contained 1:150,000,000 epinephrin since indifferent blood containing this amount caused a distinct inhibition (observation 23). That the limit must have been decidedly lower than 1:150,000,000 is shown by the fact that in reducing the concentration from 1:90,000,000 (fig. 2, observation 13), to 1:150,000,000 (fig. 1) only a moderate reduction takes place in the inhibitory effect, whereas the difference between the effect of 1:30,000,000 (fig. 2, observation 11) and 1:90,000,000 is very great. In figure 3, it is proved that indifferent blood containing a concentration of 1:200,000,000 adrenalin (observation 41) causes a much greater increase of tone of a uterus segment than the second adrenal blood specimen (observation 37), or than the third specimen (observation 39). The comparison of observations 36 and 42

shows that this uterus segment could detect a concentration of 1:300,000,000. We can conclude that the second adrenal specimen does not contain 1:200,000,000, probably not 1:300,000,000. It is certainly quite safe to assume that it could not have contained 1:250,000,000. Therefore, the output of epinephrin could not have been 0.0000035 mgm. per minute, i.e., 0.000002 mgm. per kilo of body weight per minute, or $\frac{1}{125}$ of the normal average output per kilogram, as determined by rabbit segments on shed blood. There was no evidence that any epinephrin was being given off.

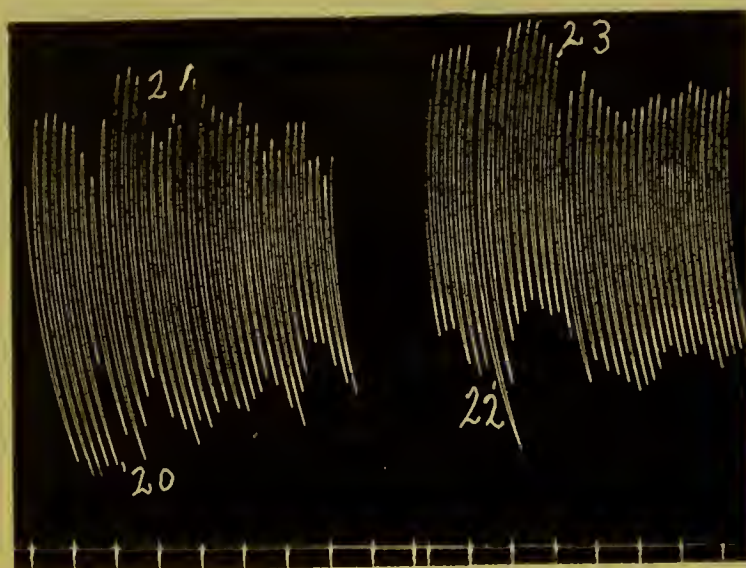


Fig. 1. Intestine tracings. Bloods from cat 90. At 20 Ringer was replaced by indifferent (arterial) blood, and this at 21 by the second adrenal blood specimen. Both bloods were diluted with three volumes of Ringer's solution. At 22 Ringer's solution was replaced by indifferent blood diluted with three volumes Ringer, and this at 23 by the indifferent blood made up with adrenalin to a concentration of 1:150,000,000, the mixture being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

Cat 108. Condensed protocol. Female. Weight, 2.75 kgm. Right adrenal excised and nerves of left adrenal cut July 6.

	WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
		Preliminary specimen	Fright	Asphyxia
	kgm.			
July 17.....	2.4	0.074		0.129
July 26.....	2.475	0.105	0.093	0.165
August 6.....		0.078	0.09	

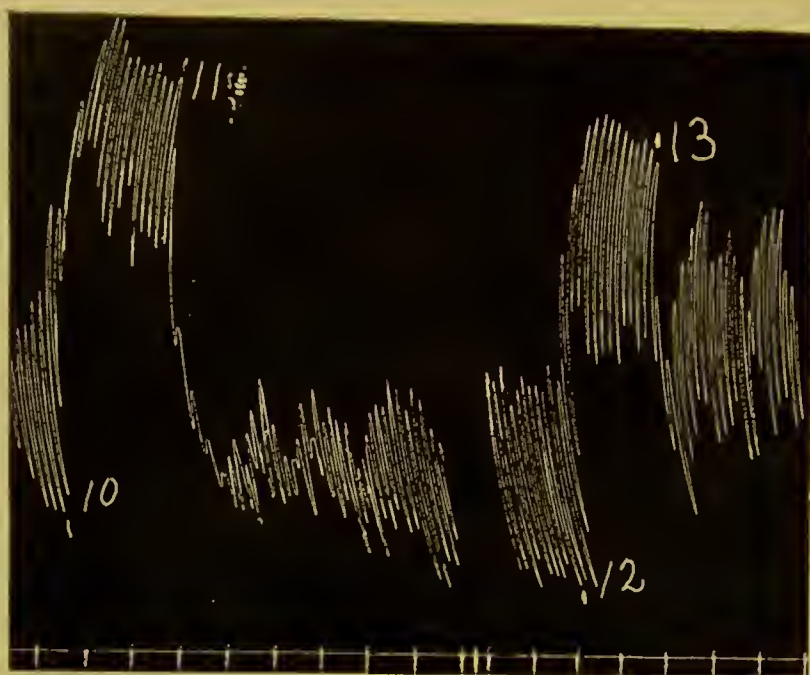


Fig. 2. Intestine tracings. Bloods from cat 90. At 10 and 12 Ringer's solution was replaced by indifferent blood diluted with three volumes Ringer, and this at 11 and 13 by the indifferent blood made up with adrenalin to a concentration of 1:30,000,000 and 1:90,000,000, respectively, the adrenalin bloods being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

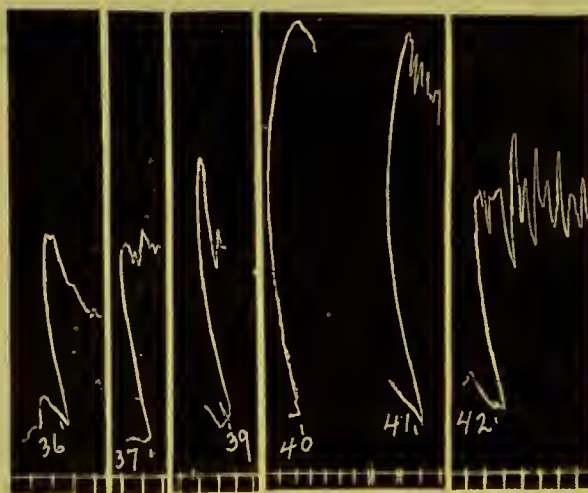


Fig. 3. Uterus tracings. Bloods from cat 90. At 36 Ringer was replaced by indifferent (arterial) blood; at 37 by the second adrenal specimen; at 39 by the third adrenal specimen; at 40 by the indifferent blood to which adrenalin had been added to make up a concentration of 1:100,000,000; at 41 by the indifferent blood to which adrenalin had been added to make up 1:200,000,000; at 42 by the indifferent blood to which adrenalin had been added to make up 1:300,000,000. All the bloods were undiluted. (Reduced to one-half.)

August 2. Excised left superior cervical ganglion.

August 10. Weight, 2.2 kgm. Made cava pocket under urethane (4 grams), tying all arterics.

1.10 p.m. Pocket experiment 4 minutes: no eye reactions.

1.16 p.m. Pocket experiment 6 minutes: no eye reactions.

1.25 p.m. 0.5 cc. adrenalin, 1:1,200,000 injected: very good eye reactions in 7.2 seconds.

1.28 p.m. 0.5 cc. adrenalin, 1:2,300,000 injected: good eye reactions in 8 seconds.

1.34 p.m. 0.5 cc. adrenalin, 1:4,500,000 injected: slight eye reactions in 10.2 seconds.

1.38 p.m. 0.5 cc. adrenalin, 1:7,000,000 injected: small retraction of nictitating in 11.4 seconds, no pupil reactions.

Now collected the following specimens of adrenal blood:

First specimen, 3.2 grams in 1 minute, blood flow 3.2 grams per minute.

Second specimen, 7.8 grams in 3.5 minutes, blood flow 2.2 grams per minute.

Third specimen, 3.0 grams in 3 minutes, blood flow 1.0 gram per minute.

Then obtained blood from abdominal aorta.

Right adrenal weighed 0.176 gram, and contains 0.23 mgm. epinephrin.

Left adrenal weighed 0.278 gram, and contained 0.26 mgm. epinephrin.

Some of the tracings of the epinephrin assay are reproduced in figures 4 to 6. In figure 4 it is shown that the second adrenal specimen (observation 4) gave no inhibition of the intestine when diluted with an equal volume of Ringer's solution. Another observation, not reproduced, proved that even when undiluted it caused no inhibition, which was also true of the third adrenal specimen (observation 14). Observation 16 (fig. 5) indicates that even the third specimen could not have contained nearly 1:200,000,000 epinephrin. Comparison of observations 10 and 16 suggests that the limit of sensitiveness of the intestine segment had not been nearly reached with a concentration of 1:200,000,000, since the inhibitory effect at 16 is far from insignificant as compared with that at 10. In figure 6 it is demonstrated by uterus tests that the second adrenal specimen (observation 21) contained less epinephrin than 1:230,000,000 (observation 20). Indeed, the effect of this sample on the uterus was not greater than that of the indifferent blood (observation 19). Taking the rate of blood flow through the adrenals during collection of the third specimen as 1 cc. per minute, it follows that the output of epinephrin per minute could not have been nearly as much as 0.000005 mgm. per minute for the animal, or 0.000002 mgm. per kilogram of body weight per minute, i.e., less than $\frac{1}{120}$ of the average output of normal cats, as determined on rabbit intestine and uterus segments. If any epinephrin at all was being given off, of which there was no evidence, the amount must have been decidedly

less than this. The eye reactions, which in this animal were very sensitive, also gave a completely negative result, even for a six-minute occlusion of the pocket. From these reactions it can also be calculated that the possible output could not have been $\frac{1}{120}$ of the normal as determined by the eye tests.

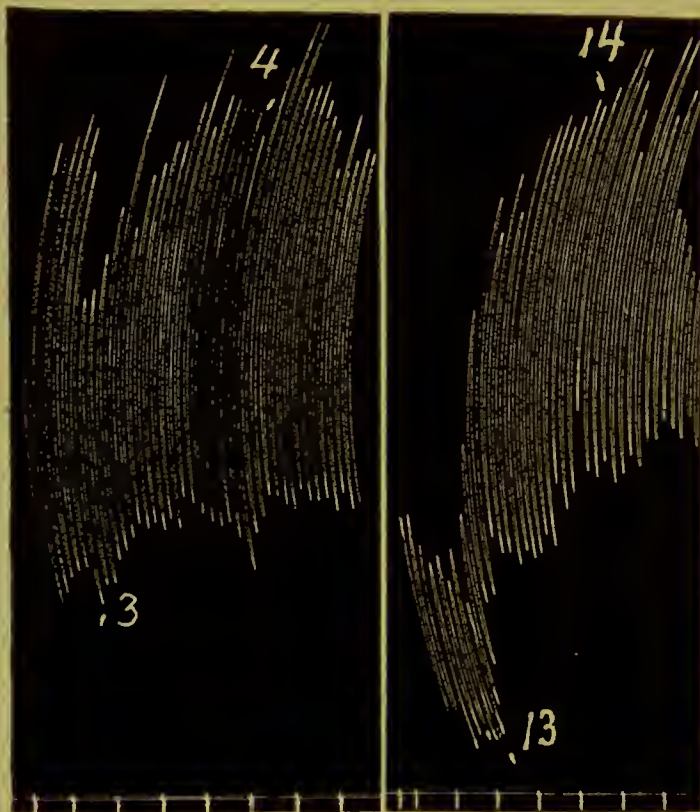


Fig. 4. Intestine tracings. Bloods from cat 108. At 3 Ringer's solution was replaced by indifferent (arterial) blood, and this at 4 by the second adrenal blood specimen. Both bloods were diluted with one volume Ringer before application to the segment. At 13 Ringer was replaced by the indifferent blood undiluted, and this at 14 by the third adrenal blood sample also undiluted. After observation 4 was completed the drum and writing point were lowered. (Reduced to two-thirds.)

Cat 92. Condensed protocol. Female. Weight, 2.525 kgm. Right adrenal excised and nerves of left cut May 9.

May 21. Weight 2.075 kgm. Blood sugar after light ether anesthesia, 0.154 per cent.

May 29. Excised left superior cervical ganglion.

June 20. Weight, 2.285 kgm., tied on board 3 minutes. Blood sugar, 0.098 per cent. After being on board 28 minutes, blood sugar, 0.108 per cent.

	WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
		Preliminary specimen	Asphyxia	Light ether
	<i>kgm.</i>			
June 18.....	2.395	0.096	0.104	
June 27.....	2.06	0.093	0.151	
July 2.....	1.96	0.064	0.092	
July 7.....	2.0	0.142*		0.176

* Very slow collection and serum separated in the pipette.

July 9. Weight, 1.93 kgm., cava pocket formed under urethane (4 grams) with all arterics tied.

12.50 p.m. Pocket experiment, 3 minutes: no eye reactions.

12.55 p.m. Pocket experiment, 5 minutes: no eye reactions.

1.05 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected; very good eye reactions in 10.2 seconds.

1.08 p.m. 0.25 cc. adrenalin, 1:1,000,000 injected: good eye reactions in 10.4 seconds.

1.10 p.m. 0.5 cc. adrenalin, 1:3,000,000 injected: small pupil; no nictitating reaction, 11.6 seconds.

1.18 p.m. 0.5 cc. adrenalin, 1:4,000,000 injected: small pupil and nictitating reaction, 10.8 seconds.

1:20 p.m. 0.5 cc. adrenalin, 1:5,000,000 injected: small pupil and nictitating reaction, 11.4 seconds.

A small specimen of adrenal blood was obtained, but not sufficient for assay, as the cat died shortly after collection was begun.

Left adrenal weighed 0.250 gram, and contained 0.19 mgm. epinephrin.

Right adrenal was crushed in removal, no assay.

In this animal the eye tests showed that a reaction could be gotten with 0.0001 mgm. epinephrin, whereas the adrenal vein blood collected for five minutes and then released produced no reaction whatever. Accordingly, the output of epinephrin could not have been as great as 0.00002 mgm. per minute, i.e., 0.00001 mgm. per kilogram of body weight per minute. This is not $\frac{1}{60}$ of the average output in normal cats, as determined by eye reactions. There was no evidence that any epinephrin was being given off.

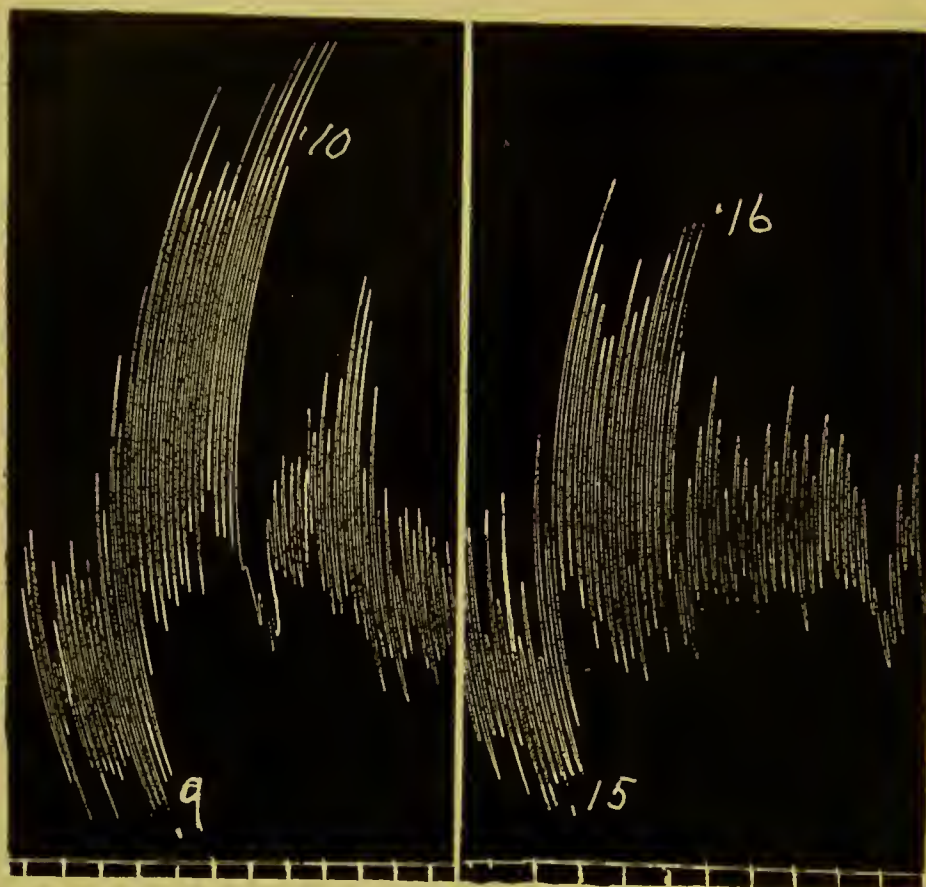


Fig. 5. Intestine tracings. Bloods from cat 108. At 9 and 15 Ringer's solution was replaced by the indifferent blood (undiluted), and this at 10 and 16 by the indifferent blood made up with adrenalin to a concentration of 1:115,000,000 and 1:200,000,000 respectively. (Reduced to two-thirds.)

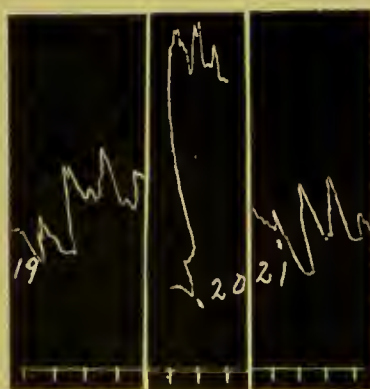


Fig. 6. Uterus tracings. Bloods from cat 108. At 19 Ringer's solution was replaced by the indifferent blood; at 20 by the indifferent blood made up with adrenalin to a concentration of 1:230,000,000; at 21 by the second adrenal specimen. All the bloods were undiluted. (Reduced to one-half.)

Cat 109. Condensed protocol. Male. Weight, 4.65 kgm. Right adrenal excised and nerves of left cut, July 6.

	WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
		Preliminary specimen	Fright	Asphyxia
	<i>kgm.</i>			
July 17.....	3.64	0.07	0.075	0.14
July 26.....	3.245	0.086	0.076	0.183

August 2. Excised left superior cervical ganglion.

August 14. Weight, 3.22 kgm., cava pocket formed under urethane (5 grams), all arteries tied.

12.26 p.m. Pocket experiment, 3 minutes: no eye reactions.

12.30 p.m. Pocket experiment, 6 minutes: no eye reactions.

0.5 cc. adrenalin, 1:2,300,000 injected: slight nictitating reaction in 11.2 seconds.

0.5 cc. adrenalin, 1:1,150,000 injected: good pupil and nictitating in 8.2 seconds.

Now collected the following adrenal blood specimens:

First specimen, 2.1 grams in 1 minute, blood flow 2.1 grams per minute.²

Second specimen, 6.9 grams in 6 minutes, blood flow 1.15 grams per minute.

Third specimen, 3.4 grams in 5 minutes, blood flow 0.7 gram per minute.

Obtained blood from abdominal aorta.

Right adrenal weighed 0.365 gram and contained 0.38 mgm. epinephrin.

Left adrenal weighed 0.331 gram and contained 0.34 mgm. epinephrin.

Some of the tracings of the epinephrin assay are given in figures 7 to 9. The second adrenal specimen diluted with three volumes of Ringer's solution (fig. 7, observation 2) gave no inhibition of the intestine, while indifferent blood containing 1:35,000,000 adrenalin similarly diluted gave a good inhibition. In figures 8 and 9 it is proved that the undiluted second adrenal blood specimen, while causing distinct inhibition of the intestine, could not have contained 1:85,000,000 epinephrin though somewhat more than 1:115,000,000. Taking the concentration as the average of these two observations, i.e., 1:100,000,000, we get 0.00001 mgm. as the output per minute or 0.000003 mgm. per kilogram of body weight per minute. This is only $\frac{1}{80}$ of the average output in normal cats, as estimated by rabbit segments in drawn blood.

² The first small sample is collected apart, in order to get rid of any epinephrin which may have been liberated by manipulation when the upper end of the pocket is being clipped off. The much greater apparent rate of blood flow sometimes seen in the collection of this sample is partly due to the inclusion of some blood already in the pocket when the clamp is applied.

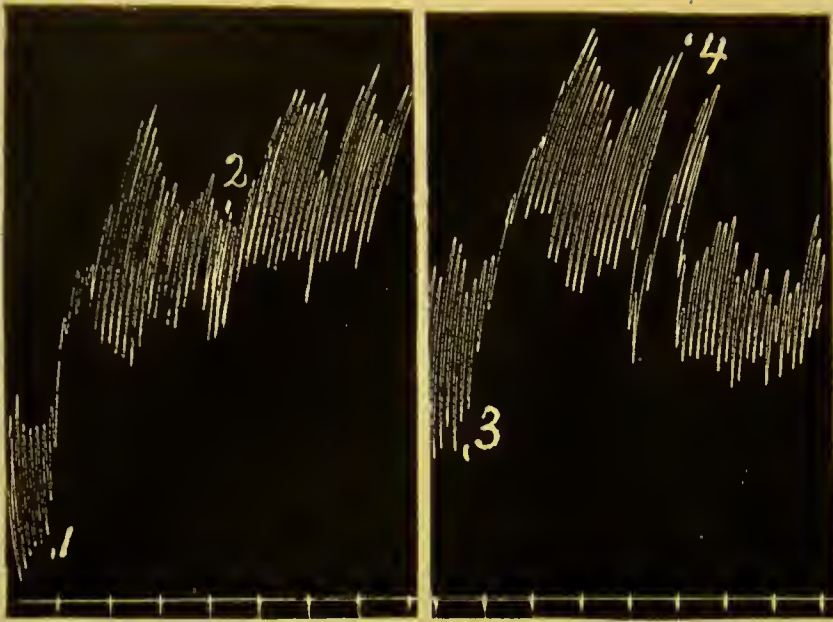


Fig. 7. Intestine tracings. Bloods from eat 109. At 1 Ringer's solution was replaced by indifferent blood and this at 2 by the second adrenal blood specimen. Both bloods were diluted with three volumes Ringer. At 3 Ringer's was replaced by the indifferent blood (diluted with three volumes Ringer), and this at 4 by the indifferent blood made up with adrenalin to a concentration of 1:35,000,000, the mixture being then diluted with three volumes Ringer before application to the segment. (Reduced to two-thirds.)

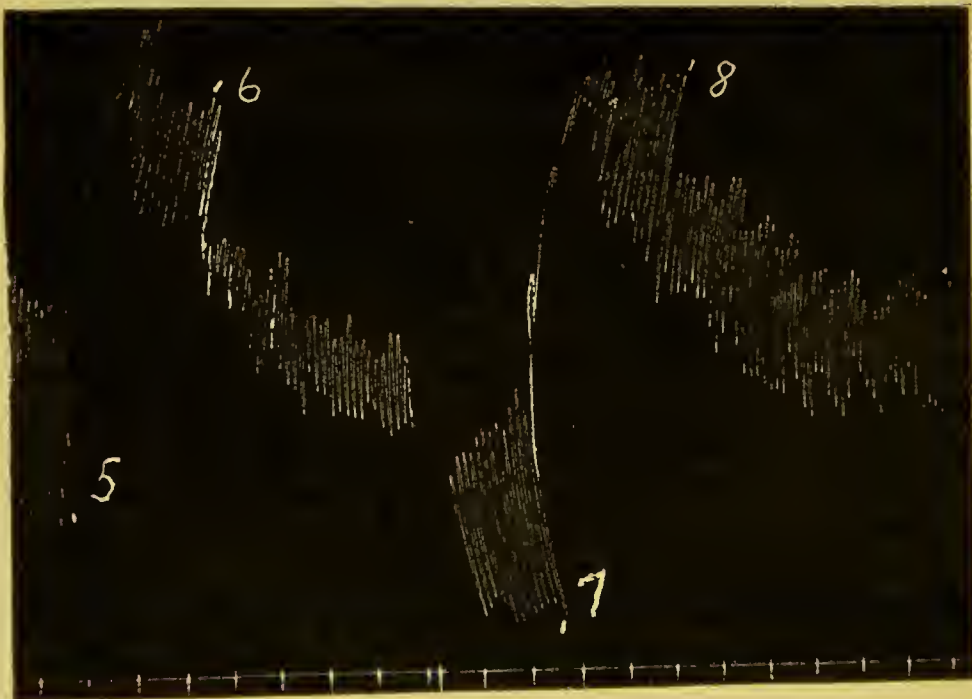


Fig. 8. Intestine tracings. Bloods from eat 109. At 5 Ringer's solution was replaced by the indifferent blood, and this at 6 by the second adrenal blood sample. At 7 Ringer was replaced by the indifferent blood, and this at 8 by the indifferent blood made up with adrenalin to a concentration of 1:115,000,000. All the bloods were undiluted. (Reduced to two-thirds.)

The eye reactions were negative even for a six-minute collection of the adrenal vein blood although a reaction was obtained when as little as 0.0002 mgm. adrenalin was injected. It can be calculated from these data that the output per kilogram of body weight per minute could not have been $\frac{1}{60}$ of the average output in normal cats, as determined by the eye reactions.

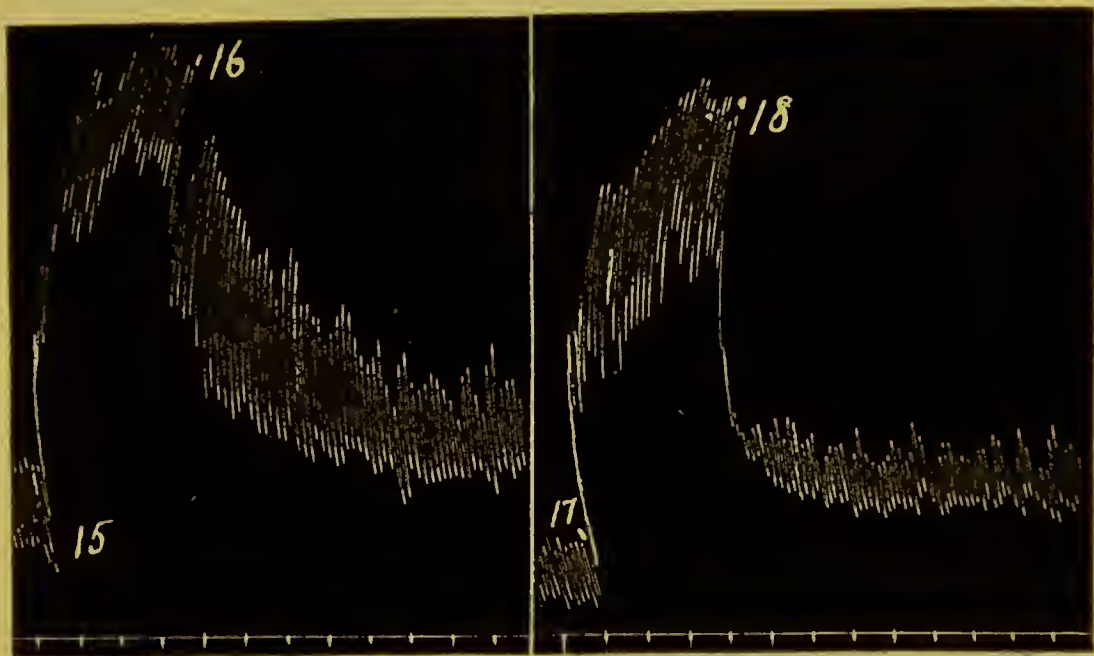


Fig. 9. Intestine tracings. Bloods from cat 109. At 15 Ringer's solution was replaced by the indifferent blood, and this at 16 by the second adrenal blood sample. At 17 Ringer was replaced by the indifferent blood, and this at 18 by the indifferent blood to which adrenalin had been added to make up 1:85,000,000. All the bloods were undiluted. The weight was less than in figure 8. (Reduced to two-thirds.)

Cat 107. Condensed protocol. Female. Weight 1.82 kgm. Right adrenal excised and nerves of left cut, July 6.

August 2. Excised left superior cervical ganglion.

	WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
		Preliminary specimen	Fright	Asphyxia
	kgm.			
July 17.....	1.5	0.075		0.247
August 6.....	1.275	0.086	0.078	0.160

August 9. Weight 1.27 kgm. Under urethane (3 grams) cava pocket formed with all arteries tied. Positive pupil reactions were obtained on collecting adrenal vein blood in the cava pocket and then releasing it, but there was no movement of the nictitating membrane. The following samples of adrenal blood were collected.

First sample, 1.1 grams in 1 minute, blood flow 1.1 grams per minute.

Second sample, 3.8 grams in 5 minutes, blood flow 0.8 gram per minute.

Third sample, 3.3 grams in 7 minutes, blood flow 0.47 gram per minute.

Indifferent blood was obtained from the abdominal aorta.

Right adrenal weighed 0.168 gram and contained 0.23 mgm. epinephrin.

Left adrenal weighed 0.152 gram and contained somewhat more than 0.12 mgm. epinephrin.

Figure 10, in which a few of the tracings of the epinephrin assay are reproduced, shows that even the third adrenal specimen, in spite of the relatively small flow of blood during its collection, had scarcely 1:35,000,000 epinephrin and much less than 1:17,000,000. The epinephrin output was therefore less than 0.000015 mgm., i.e., about 0.00001 mgm. per kilogram of body weight per minute. This is not more than $\frac{1}{25}$ of the average output of normal cats per kilogram. It is only because of the slow blood flow that the concentration is even as great as 1:35,000,000.

Cat 91. Condensed protocol. Female. Weight, 2.23 kgm. Right adrenal excised and nerves of left cut, May 9.

May 29. Weight 2.00 kgm., dextrose in blood, 0.105 per cent.

May 29. Excised left superior cervical ganglion.

June 20. Weight, 1.95 kgm. Tied on board 3 minutes, blood-sugar, 0.108 per cent. Still tied on board 12 minutes, blood-sugar, 0.108 per cent.

	WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
		Preliminary specimen	Light ether	Asphyxia
	<i>kgm.</i>			
June 21.....	2.0	0.092	0.98	
July 2.....	2.145	0.077		0.10
July 7.....	2.05	0.116		0.321

July 9. Weight 2.065 kgm. Under urethane (4 grams) cava pocket formed with all arteries tied.

4.23 p.m. Pocket experiment, 2 minutes: slight pupil reaction in 9 seconds.

4.25 p.m. Pocket experiment, 2 minutes: slight pupil reaction in 11.6 seconds.

4.28 p.m. Pocket experiment, 4 minutes: small pupil and nictitating reaction in 10 seconds.

4.35 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected: good pupil, slight nictitating in 9.2 seconds.

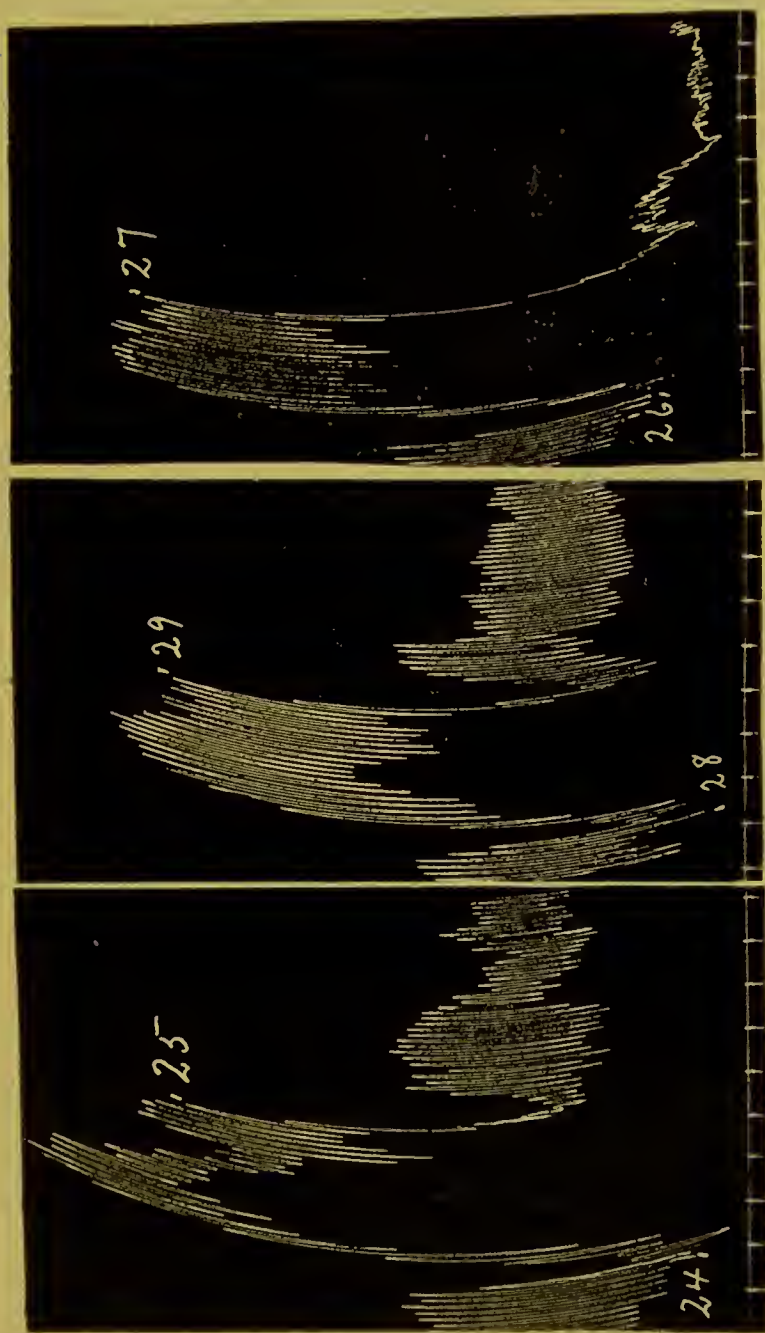


Fig. 10. Intestine tracings. Bloods from cat 107. At 24 Ringer was replaced by indifferent (arterial) blood, and this at 25 by the third adrenal blood sample. Both bloods were diluted with one volume of Ringer. At 26 Ringer was replaced by the indifferent blood diluted with an equal volume of Ringer and this at 27 by the indifferent blood made up with adrenalin to 1:17,000,000, and then diluted with an equal volume of Ringer. At 28 Ringer was replaced by the indifferent blood diluted with an equal volume of Ringer, and this at 29 by the indifferent blood made up with adrenalin to a concentration of 1:35,000,000 and then diluted with an equal volume of Ringer. (Reduced to two-thirds.)

- 4.40 p.m. 0.5 cc. adrenalin, 1:2,000,000 injected: small pupil in 12.2 seconds (like observation at 4.28).
4.45 p.m. Pocket experiment, 4 minutes; pupil reaction in 8.2 seconds, greater than at 4.40, more like that at 4.35.
4.48 p.m. 0.5 cc. adrenalin, 1:1,000,000: slight pupil reaction in 12.4 seconds.
4.57 p.m. Pocket experiment, 2 minutes: small pupil reaction in 8.6 seconds.
5.00 p.m. 0.5 cc. adrenalin, 1:1,000,000 injected; good pupil reaction in 10.8 seconds (larger than in observation at 4.57).
5.07 p.m. Pocket experiment, 3 minutes; pupil reaction nearly the same as that in observations at 5.00.

A number of additional observations on the eye reactions with adrenalin injection, and adrenal vein blood released from the pocket were made. Then the following specimens of adrenal blood were drawn through a cannula in the cava and tested on rabbit intestine and uterus segments.

First specimen, 1.1 grams in 2 minutes, blood flow, 0.55 gram per minute.

Second specimen, 2.5 grams in 15 minutes, blood flow 0.17 gram per minute.

Third specimen, 1.8 grams in 23 minutes, blood flow 0.08 gram per minute.

The blood flowed very slowly.

Right adrenal weighed 0.232 gram and contained 0.26 mgm. epinephrin.

Left adrenal weighed 0.265 gram and contained somewhat more than 0.16 mgm. epinephrin.

In figures 11 and 12 are reproduced some of the tracings of the epinephrin assay. It gave much the greatest concentrations of epinephrin in the adrenal vein seen in the series of cats whose adrenal innervation had been interfered with. The second adrenal specimen had a concentration of epinephrin about equal to 1:1,500,000 (fig. 11). It was distinctly greater than 1:2,000,000. In the third specimen the concentration was greater than 1:800,000, and less than 1:500,000 (fig. 12). The blood had an unusually high proportion of plasma, not far from 90 per cent, and the flow during collection was very small. For both reasons the concentration is high. We have stated elsewhere (3), (6) that a concentration of more than 1:1,000,000 is unusual in cats' adrenal vein blood, but the proportion in serum can be considerably higher since the erythrocytes contain practically no epinephrin. The output of epinephrin calculated for the second adrenal specimen was 0.0001 mgm. per minute for the animal and 0.00005 mgm. per kilogram per minute, or about $\frac{1}{5}$ of the normal output in the cat as determined on drawn blood by rabbits' intestine segments. Taking the concentration in the third specimen at 1:650,000, we get 0.00012 mgm. per minute, or 0.00006 mgm. per kilogram of body weight per minute.

The eye reactions gave about 0.00015 mgm. per minute, or 0.00007 mgm. per kilogram of body weight per minute as the output, i.e., about $\frac{1}{8}$ or $\frac{1}{10}$ of the normal as determined by the eye tests.

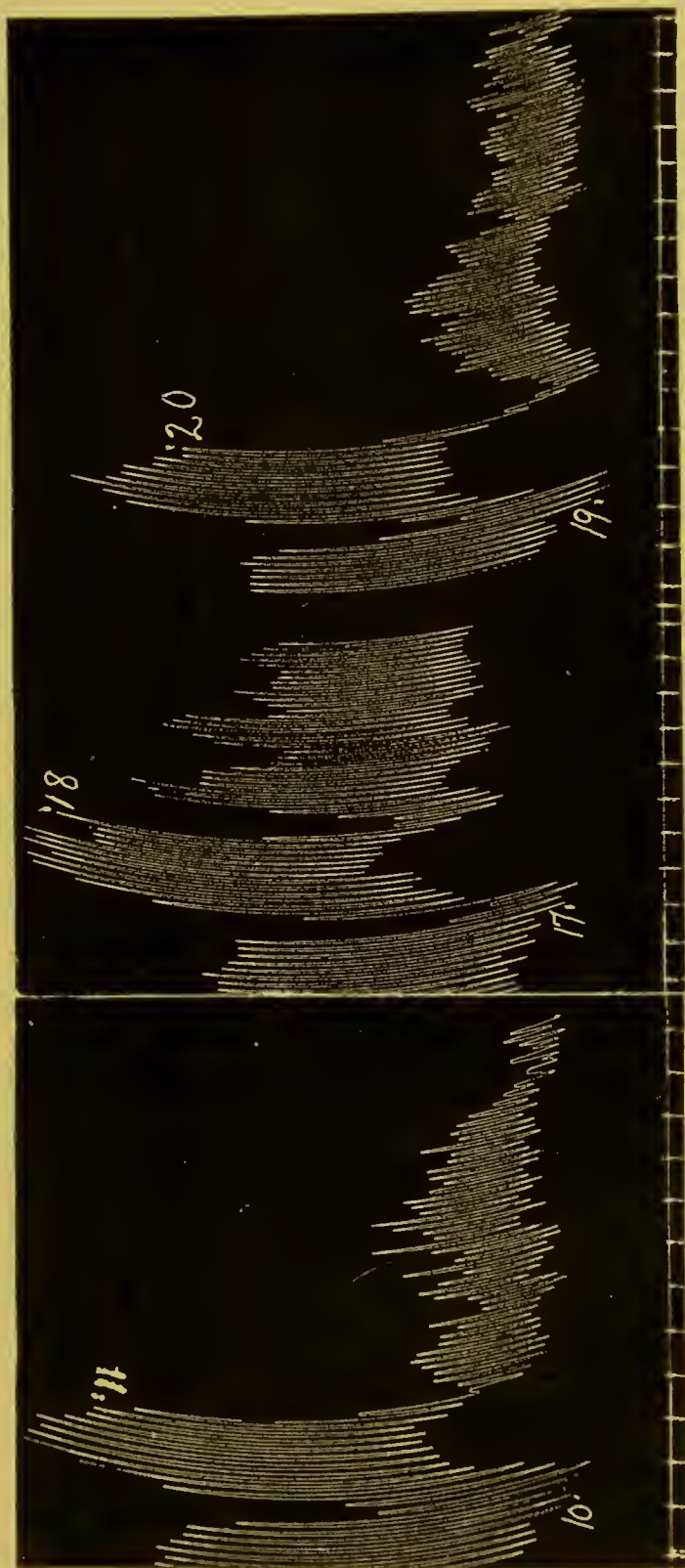


Fig. 11. Intestine tracings. Bloods from cat 91. At 10 and 17 Ringer's solution was replaced by indifferent (arterial) blood, diluted with five volumes Ringer, and this at 11 and 18 by the indifferent blood made up with adrenalin to concentrations of 1:1,500,000 and 1:2,000,000 respectively, the adrenalin bloods being then diluted with five volumes Ringer before application to the segment. At 19 Ringer's solution was replaced by the indifferent blood and this at 20 by the second adrenal blood specimen, both bloods being diluted with five volumes Ringer. (Reduced to two-thirds.)

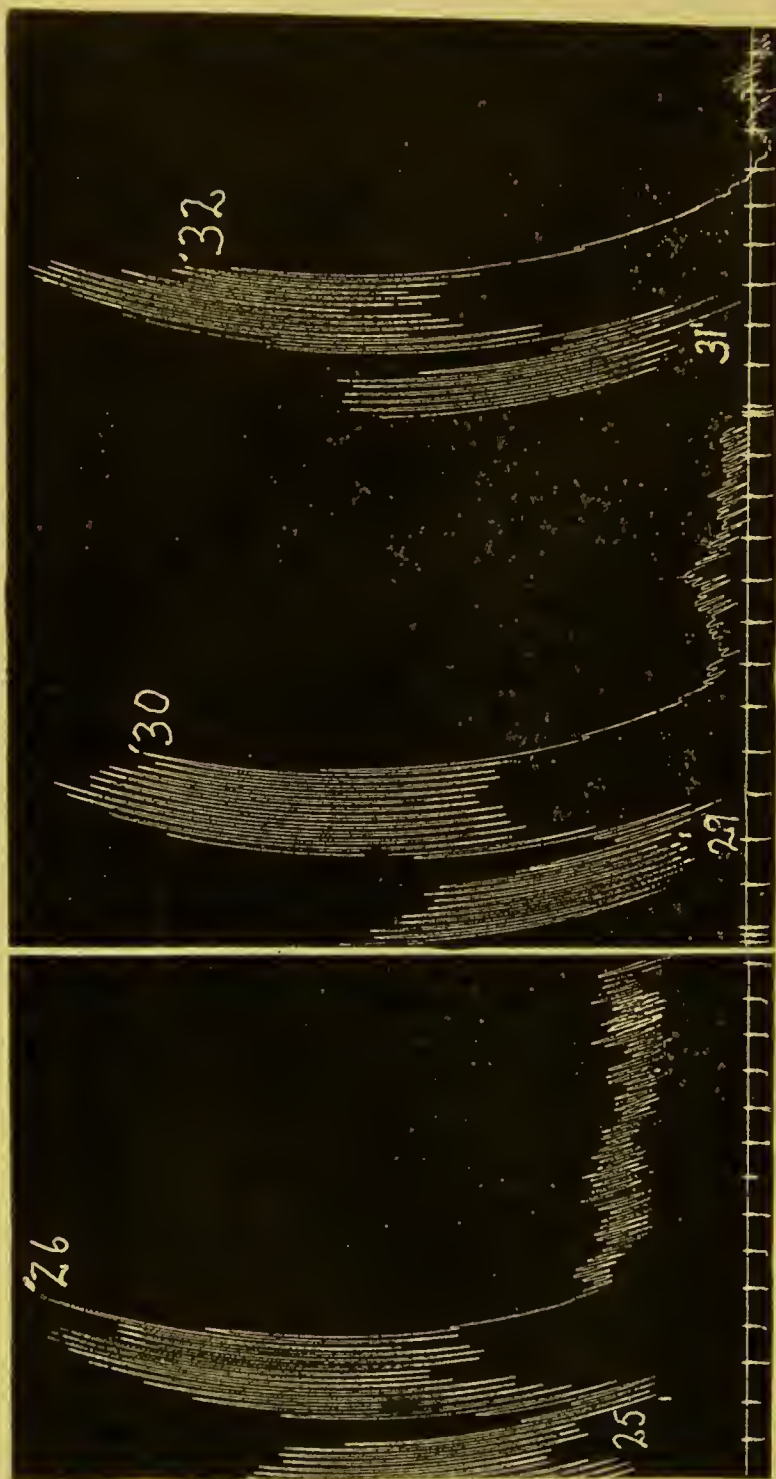


Fig. 12. Intestine tracings. Bloods from cat 91. At 25 and 31 Ringer's solution was replaced by the indifferent blood diluted with five volumes Ringer, and this at 26 and 32 by the indifferent blood made up with adrenalin to concentrations of 1:800,000 and 1:500,000 respectively, the adrenalin bloods being diluted with five volumes Ringer before application to the segment. At 29 Ringer's solution was replaced by the indifferent blood and this at 30 by the third adrenal blood sample, both bloods being diluted with five volumes Ringer. In observation 32 the writing point went below the drum and stayed down for a time. (Reduced to two-thirds.)

It is commonly stated that in the rabbit the left splanchnic is much more important than the right for the production of some of the experimental hyperglycemias. Although there is no reason to believe that such a difference exists in the cat, the left adrenal was removed in one of the animals (cat 121) and the nerves of the right adrenal, including of course the right splanchnic, cut. The results differed in no way from those obtained on the other cats.

Cat 121. Condensed protocol. Female. Weight, 1.975 kgm. Left adrenal excised and nerves of right cut, July 31.

August 13. Weight, 1.71 kgm. Preliminary blood specimen, 0.111 per cent dextrose. Asphyxia, 0.163 per cent dextrose.

August 16. Excised left superior cervical ganglion.

August 24. Weight, 1.70 kgm. Under urethane (3 grams) cava pocket formed, with all arteries tied.

11.35 a.m. Pocket experiment, $3\frac{1}{2}$ minutes: no eye reactions.

11.40 a.m. Pocket experiment, 6 minutes: no eye reactions.

11.50 a.m. 0.5 cc. adrenalin, 1:1,150,000: excellent eye reactions in 11.8 seconds.

0.5 cc. adrenalin, 1:2,300,000: good eye reactions in 17.4 seconds.

Now collected the following specimens of adrenal vein blood:

First specimen, 0.8 gram in 1 minute, blood flow 0.8 gram per minute.

Second specimen, 3.6 grams in $6\frac{1}{2}$ minutes, blood flow 0.5 gram per minute.

Third specimen, 5.2 grams in 11 minutes, blood flow 0.47 gram per minute.

Fourth specimen, 2.0 grams in 6 minutes, blood flow 0.33 gram per minute.

During collection of the fourth specimen, a clot had to be squeezed out of the pocket.

Right adrenal weighed 0.242 gram and contained 0.26 mgm. epinephrin.

Left adrenal weighed 0.152 gram and contained 0.16 mgm. epinephrin.

It was shown on the rabbit intestine and uterus segments that the third adrenal blood specimen contained about 1:30,000,000 epinephrin. The rate of blood flow was approximately 0.45 cc. per minute. Accordingly the output of epinephrin per minute was about 0.000015 mgm. per minute for the animal or 0.00001 mgm. per kilogram of body weight per minute, i.e., $\frac{1}{25}$ of the average normal output, as determined on drawn blood with rabbits' segments. The eye reactions were negative even for a six-minute collection although 0.0002 mgm. of adrenalin gave a reaction which was more than minimal. The output of epinephrin was therefore not above 0.000035 mgm. per minute for the animal (about $\frac{1}{45}$ of the normal average), or 0.00002 mgm. per kilogram of body weight per minute, (about $\frac{1}{30}$ of the normal average), as determined by eye reactions.

As regards the blood-sugar percentages, it will be seen from the protocols that asphyxia and etherization caused distinct hyperglycemia in the cats whose epinephrin output had been abolished, within the

limits of detection by the methods employed as well as in those in which a small residual output was still present. If the results on these cats are compared with those obtained in the same way on normal cats (tables 4 and 5) it will be seen that no essential difference can be made out. The precise amount of the hyperglycemia has no particular significance for it is well known that this is influenced greatly by the nutritive condition of the animals, particularly by the glycogen content of the liver. The same is true of the occasional failure to obtain the expected hyperglycemia, which is seen in the normal animals as well as in those subjected to the adrenal operation. It is conceivable that in the operated cats the loss of one major splanchnic, in addition to other sympathetic fibers, possibly going to the liver, if these nerves are at all concerned in the hyperglycemias studied, may have prevented the percentage of dextrose in the blood from rising as high after asphyxia or etherization as in the normal cats, although it is not cer-

TABLE 4

NUMBER OF CAT	DATE	BODY WEIGHT	PERCENTAGE OF BLOOD-SUGAR		
			Preliminary specimen	Fright	Asphyxia
		<i>kgm.</i>			
111 {	July 19*	2.4			
	July 30	2.285	0.082	0.098	0.134
	August 13	2.11	0.108		0.225
112 {	July 19*	2.24			
	July 30	2.015	0.097	0.105	0.282

* This was the date of the operation.

tain from the results that there is any decided deficiency in this respect in the former group.

In table 4 are given some observations on two cats in which a dummy operation was performed, as already mentioned, to imitate as far as possible the nutritive consequences of the adrenal operation, except that in these cats the adrenals were intact.

In table 5 are displayed the results of the blood-sugar estimations on a series of normal cats subjected to the same procedures as the cats which had undergone the adrenal operations.

From these results it seems impossible to draw any other conclusion than that the hyperglycemia associated with asphyxia and with etherization is not produced through the intervention of epinephrin liberated from the adrenal glands, or at least that the liberation of epi-

TABLE 5

NUMBER OF CAT	DATE	BODY WEIGHT	PERCENTAGE OF DEXTROSE IN BLOOD			
			Normal or preliminary sample	Fright	Ether	Asphyxia
		<i>kgm.</i>				
5	May 18	2.36	0.109			
	June 12	2.065				0.163
	June 27	2.43	0.096*			0.321
6	May 19	3.065			0.332†	
	June 16	2.124‡				0.422-A§ 0.510-B
8	June 1	2.585		0.11		
9	May 22	2.815				0.226
10	May 22	2.53			0.277	
17	June 27	2.68	0.089▪		0.157	
	July 18	2.50	0.100			0.257
	June 18	2.97	0.131	0.171		
21	July 3	2.89	0.078			0.079
	July 18	2.865	0.122			0.201
	June 18	2.775	0.109			0.128
22	June 20	2.70	0.115-A• 0.101-B		0.161	
	July 3	2.525	0.080			0.096
23	July 18	1.910	0.081	0.157		0.214
24	July 24	2.140	0.086	0.104		0.329
26	July 3	2.630	0.095			0.214
27	July 24	2.445	0.104	0.10		0.306
	July 3	2.885	0.079			0.109
	July 21	2.745	0.100	0.107		0.201
	August 1	2.585	0.090	0.079		0.214
30	August 1	2.280	0.071		0.123	

* Struggled and micturated during collection of normal specimen.

† The ether was too concentrated (in jar); artificial respiration had to be performed.

‡ The animal had distemper and had lost weight.

§ Blood was obtained with difficulty; finally the femoral artery was cut and sample A collected into 2 per cent oxalate (without laking), and B into water (well laked).

|| These cats were kept tied on the board during the period of frightening; the others were placed in a cage.

▪ Very much excited during collection of normal specimen.

• A, collected immediately after the animal was tied on the board; B, after it had been tied for eight minutes on the board.

nephrin is not essential to the production of the hyperglycemia. The question whether these experimental hyperglycemias depend upon nervous influences exerted upon the liver so as to hasten the transformation of glycogen into dextrose, or upon a more direct influence, e.g., through changes in the hydrogen-ion concentration of the blood (7) does not concern us here. In one experiment (cat 113) after the right adrenal had been excised and the nerves of the left cut, as in the ordinary operation, the right major splanchnic was also divided after an interval of twenty-seven days. Fourteen days after the second operation, the animal being in excellent condition, the preliminary blood sample collected while the cat was quiet contained 0.089 per cent of dextrose. A second sample, taken after the animal had been frightened by a dog for thirty-eight minutes, contained 0.084 per cent. Urine passed two to three minutes after the end of the frightening period gave a negative test for sugar. A third blood sample obtained after fifteen minutes of asphyxia, immediately succeeding the period of fright, contained 0.153 per cent. The asphyxia hyperglycemia was therefore unmistakably produced in this animal after section of both splanchnics. However, our problem was merely to determine whether those hyperglycemias could or could not be produced in the absence of epinephrin. If epinephrin is not necessary for the marked and prompt augmentation of the blood-sugar observed in the conditions studied, then the deductions which have been drawn as to the importance of the epinephrin secretion of the adrenals in the mobilization of sugar can no longer be upheld.

We desire to make it clear that our present conclusions concern solely the hyperglycemia produced by asphyxia and etherization in cats. Whether such a hyperglycemia as that produced by piquê, in which according to the best evidence the nervous system is essentially concerned, can also be elicited in the absence of epinephrin we have not as yet sufficient data to decide. The hypoglycemia described by certain observers after adrenalectomy was not seen in our animals. The average for 19 "normal" or preliminary blood samples from the control cats was 0.096 per cent. of dextrose. The average for 19 similar samples from the cats which had been subjected to the adrenal operation was the same (0.095 per cent.).

SO-CALLED EMOTIONAL HYPERGLYCEMIA

A question which has been much discussed and which cannot very well be avoided in work on the blood-sugar, is the influence of emotional

disturbances upon the sugar content. Some writers (8) have convinced themselves that emotional hyperglycemia is so easily produced in the ordinary laboratory animals that it is impossible to obtain "normal" sugar percentages unless great precautions are adopted to prevent excitement. Others (9) have found it difficult to convince themselves that a real emotional hyperglycemia exists. We have no desire to enter into this question except insofar as it arises out of our own work. The frightening experiments were merely incidental, advantage being taken, as already mentioned, of opportunities afforded by animals which were particularly quiet during the withdrawal of the preliminary blood specimen, to see whether any marked or constant difference would be found when they were then frightened, usually by a barking dog. Since the adrenals have been supposed by some observers to be concerned also in the production of emotional hyperglycemia, we made such experiments not only on normal cats but also on cats which had undergone the adrenal operations. As will be seen from the results given in the protocols and tables, we have been unable to demonstrate in normal animals any constant increase in the percentage of sugar in the blood which could be considered as associated with emotional excitement. Further, our experiments do not reveal any essential difference between the results of emotional excitement on the blood-sugar in the normal and in the operated cats. We do not claim that our observations disprove the existence of a true emotional hyperglycemia, but they do suggest that if it exists it is a rather infrequent phenomenon, not to be elicited at will, in cats at least, as the asphyxia hyperglycemia can be elicited and so insignificant in amount that very numerous observations would be necessary to disentangle it from the uncontrollable variations in the sugar content.

The possibility must be admitted that different species of animals, perhaps different individuals of the same species, may vary in their susceptibility to emotional excitement in this regard. If this were so, man might be expected to be more susceptible than lower animals. On the other hand, great differences in the effects of such conditions as asphyxia and ether anesthesia would scarcely be looked for. However this may be, a survey of the data in the literature as well as our own data, indicates that there is a fundamental distinction between asphyxial and post-anesthetic hyperglycemias, which are well established and easily verifiable, and "emotional hyperglycemia," the existence of which is asserted by some authors on the basis of small and inconstant differences in the blood sugar content, which other writers consider to fall within the limits of variation of the normal. As regards any relation between the epinephrin discharge and emotional hyperglycemia, even granting the existence of the latter,

our experiments seem to show that there is no such association. The so-called "Fesselungs" glycosuria and hyperglycemia perhaps stand on a different footing from emotional glycosuria and hyperglycemia; for there are several factors besides the emotional one which might possibly affect the blood-sugar content of an animal tied down in an abnormal position and struggling to free itself. Such data as have been incidentally accumulated by us on this point seem to indicate a real increase in the percentage of blood-sugar when the animal has been kept for some time on the board, whether or not it has been purposely "frightened." However, we do not desire to lay stress on these observations, since where the maximum changes in the sugar content are small large numbers of experiments would be necessary to reach a safe conclusion. The three cats included in table 6 had only recently come to the laboratory. If this circumstance had anything to do with the result, we should be inclined to find the explanation rather in their nutritive condition than in greater susceptibility to excitement on account of the strangeness of their surroundings. For cats which

TABLE 6

NUMBER OF CAT	DATE	TIME ON BOARD	DEXTROSE	REMARKS
		<i>minutes</i>	<i>per cent</i>	
23	{ June 19	3	0.178	Cried during puncture of vein
		20	0.216	Cried during puncture of vein
24	{ June 19	8	0.079	Excited
		22	0.135	Excited
25	{ June 19	3	0.131	Excited
		13	0.164	Excited

were to all appearance much more strongly excited, but were not kept tied down, showed no definite increase in the blood-sugar.

Observations on animals which were frightened while tied on the board are given elsewhere in the paper (table 5). Where we wished to study the effect of emotional excitement alone, the animals were set free after the preliminary specimen of blood had been obtained, and were frightened in a cage.

IS THE OUTPUT OF EPINEPHRIN INCREASED IN NORMAL ANIMALS BY CONDITIONS WHICH CAUSE OR HAVE BEEN SUPPOSED TO CAUSE HYPERGLYCEMIA?

If the hyperglycemias studied do not depend upon epinephrin, the attempts which have been made to show that the conditions associated with the increased blood-sugar content also are associated with increased output of epinephrin would, even if they were successful, have

no bearing upon the mechanism or significance of the hyperglycemias in question. Even if it were demonstrated that asphyxia, etc., caused a marked increase in the epinephrin output, this could not be used as an argument in favor of the theory that it is a function of the adrenals to aid in the mobilization of sugar, once it was shown that this mobilization could occur all the same in the absence of epinephrin. We have, however, failed to demonstrate any definite increase in the output of epinephrin either through asphyxia or through stimulation of sensory nerves (10).

As for emotional disturbance, no method of satisfactorily testing the question has occurred to us. General anesthesia, of course, cannot be employed and the collection of the unmixed adrenal vein blood is therefore impracticable. The method adopted by Cannon and de la Paz could at most yield information as to the concentration of epinephrin in the inferior cava blood. They do not seem to have made any assay of the concentration in their published work; but the concentration alone, unless the amount of blood passing the point of collection in a given time is known, does not permit the rate of liberation of epinephrin to be calculated. Nevertheless, in order to have first-hand experience of the method we repeated the observations of Cannon and de la Paz in three cats, imitating in every particular their procedure, except that the blood specimens were tested on rabbits' intestine and uterus segments instead of cats' intestine strips. Cannon and Hoskins in their work on asphyxia and sensory stimulation used rabbits' intestine segments for the tests, and state that they are not inferior to cats' intestine strips.

In testing the bloods we did not empty the cylinder when replacing one liquid by another, as apparently Cannon and his associates do, for this produces a distortion of the curve at the critical point owing to the weight of the segment coming on the lever when the cylinder is emptied, and coming off the lever when the new liquid is introduced. A further objection to this method is that a segment which has been immersed in a liquid of given temperature saturated with oxygen is suddenly exposed to air, and then again suddenly immersed in another liquid. This can hardly be done without altering the temperature and oxygenation of the segment, and artificial effects including inhibition may sometimes be produced in this way. As in all our previous work, the contents of the cylinder were changed by displacement from below up, the new liquid, previously well oxygenated, being run in gently from a pipette drawn out to a fine point, and the old liquid overflowing into the bath. The pointed end of the pipette is bent at about a right angle so that it is easily introduced to the bottom of the cylinder without disturbing the preparation. The same quantity of liquid is always introduced when comparative observations are

being made, the pipette being filled to a mark. The amount of admixture with the liquid which is being displaced is small and approximately constant; and all that is necessary to eliminate any error due to admixture, and to make the liquid in the cylinder precisely the same as that in the stock from which it is taken, is to run in a sufficient excess of the liquid beyond the amount corresponding to the capacity of the cylinder. The curve is unaffected by the changing of the liquid in the cylinder except insofar as it produces a physiological alteration in the segment. Unless the point at which the change is made is marked on the tracing, it would be impossible to identify it. If it is desired to change the liquid by drawing off the contents of the cylinder, which would be advantageous when it is necessary to save them, this should be managed so that the new liquid enters the cylinder at the same rate as that at which the old liquid is withdrawn.

Cat 25. Condensed protocol. Female. Weight, 2.475 kgm. The cat was secured on a comfortable holder of the same kind as that used by Cannon for his work with the Roentgen rays on the movements of the alimentary canal. Under local anesthesia with ethyl chloride, the left femoral vein was exposed. A flexible catheter oiled inside and outside was introduced with its opening at a level 5 to 6 mm. anterior to the orifices of the adrenal veins. The level of the catheter was verified at the end of the experiment. While it could not be said that the animal showed no discomfort, the degree of disturbance during the "quiet" period was no doubt much less than when the cat was frightened by a barking dog. Specimens of blood were obtained through the catheter with the aid of an aspirator, as follows:

1. While the cat was quiet.
2. After $1\frac{1}{2}$ minutes excitement by the dog.
3. After 3 minutes excitement by the dog.
4. After 13 minutes excitement by the dog. The cat micturated.
5. After 15 minutes rest while the animal was fairly quiet.

The sixth catheter specimen was obtained with the catheter inserted so that its orifice was about 8 cm. below the adrenals. This specimen was taken fifteen minutes after the fifth catheter specimen. A sample of blood was then obtained from the femoral vein through a cannula, and another sample from the carotid artery. The bloods were defibrinated and tested on rabbit intestine and uterus segments. Combined weight of adrenals, 0.38 gram.

Figures 13 to 15 show some of the tracings. Those in figure 13 afford no evidence that blood collected at the level of the adrenals during excitement caused any inhibition of the rabbit intestine segment when it displaced blood collected at the same level with the animal quiet. The bloods were tried both undiluted (observation 2) and diluted with Ringer's solution (observation 27). In figure 14, blood from the same level obtained after a period of great excitement, during which the animal micturated, was caused to displace blood obtained while the animal was quiet (observation 8). Instead of inhibition there was a further increase of tone. This was not due to the so-called "sthenic"

effect of a small concentration of epinephrin, for a similar increase of tone was seen when the "quiet" specimen replaced the "excited" specimen (observation 10). In figure 15, blood collected during excitement from the adrenal level produced also some increase of tone instead of inhibition when it replaced indifferent venous blood from the lower part of the inferior cava (observation 48). The effect was not essentially different from that caused by blood collected from the adrenal level after a period of quiet when it replaced blood previously drawn during excitement (observation 46). The result of this experiment, then, was negative, the adrenal vein blood, which doubtless contained epinephrin, being too much diluted by the general mass of epinephrin-free blood in the cava to yield reactions with the intestine segments (10), (11), (12).

It is surprising how little account has been taken by many writers on this subject of the great dilution which any epinephrin liberated from the adrenals must undergo before it reaches the systemic capillaries and veins, apart from loss due to its oxidation or removal. A little reflection on this point would have made the concentrations of epinephrin sometimes alleged to have been found in venous blood incredible, because they would involve incredible concentrations in the blood leaving the adrenals. In a quite recent paper, e.g., Herring (13) quotes A. Fraenkel (14) and Broking and Trendelenburg (15) as having shown that in Graves' disease the epinephrin content of the blood obtained from an arm vein is increased, as compared with the normal blood. Now Fraenkel reports that he found



Fig. 13. Intestine tracings. Bloods collected by catheter from cat 25. At 1 Ringer's solution was replaced by the first catheter specimen, from the level of the adrenals, collected while the cat was quiet, and this at 2 was replaced by the second catheter specimen collected at the same level after one and one-half minutes of excitement. Both bloods were undiluted. At 26 Ringer was replaced by the first catheter specimen (quiet) and this at 27 by the third catheter specimen, collected after three minutes of excitement. Both bloods were diluted with four volumes Ringer. (Reduced to two-thirds.)

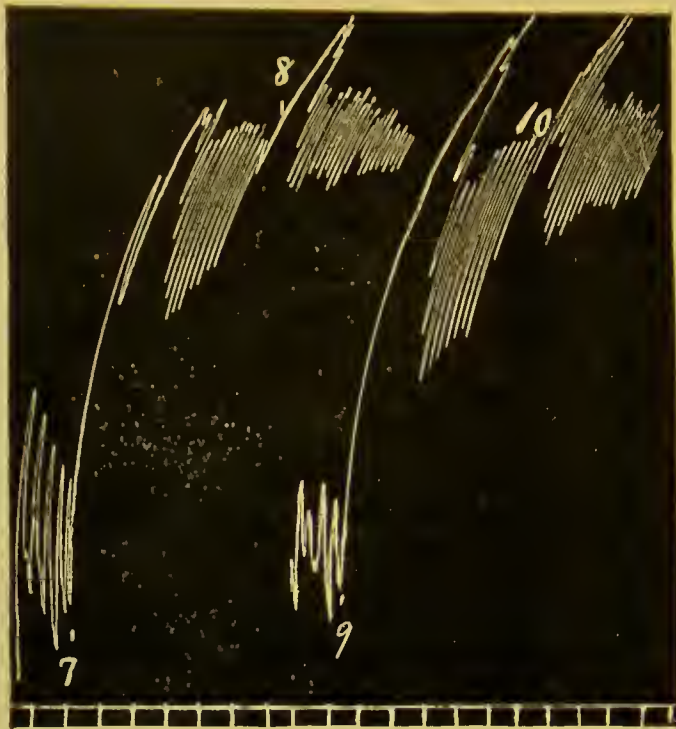


Fig. 14. Intestine tracings. Bloods from same cat used in figure 13. At 7 Ringer's solution was replaced by the first catheter specimen (quiet), and this at 8 by the fourth catheter specimen collected at the adrenal level after thirteen minutes of great excitement. At 9 Ringer was replaced by the fourth catheter specimen, and this at 10 by the first catheter specimen. (Reduced to two-thirds.)



Fig. 15. Intestine tracings. Bloods from same cat as in figures 13 and 14. At 45 Ringer was replaced by the fourth catheter specimen (excitement) and this at 46 by the fifth catheter specimen (collected without excitement). At 47 Ringer was replaced by blood from the lower part of the inferior vena cava, and this at 48 by catheter blood collected at the adrenal level after ten minutes excitement. (Reduced to two-thirds.)

in a case of Graves' disease a concentration of 1: 400,000 in the venous blood. How can a statement of this sort, which according to the best available evidence would imply a concentration in the adrenal vein blood of probably at least 1: 1000 or 1: 2000 be seriously accepted? In cats under experimental conditions which are widely believed to increase the output of epinephrin, a concentration of more than 1: 1,000,000 is rarely seen in the blood coming from the adrenals. In this connection it must be remembered that although some of the blood which passes through the adrenal cortex may come into relation with the cells of the medulla before issuing from the gland, it will be safe to assume that the amount of blood which can have effective interchange with the medulla is only a fraction of the total adrenal blood flow. The concentration of epinephrin in the blood of the medullary sinusoids must therefore be higher, perhaps much higher, than that in the blood of the adrenal veins. Trendelenburg (16) has since come to the conclusion that the method of Laewen (frog perfusion), as he applied it in his earlier work, is unreliable because of the rapid development of pressor substances in shed blood, and he does not now believe that the arterial blood (in rabbits) can contain even 1: 1,000,000,000 or 1: 2,000,000,000 epinephrin. Fraenkel states that he was able to detect epinephrin (1: 20,000,000 or more) in *all* specimens of *normal* human blood! Fraenkel's data are absolutely worthless, as he ignored the fact that it was the serum and not epinephrin in it, which produced the effects on the rabbit uterus segments, which he relied upon for estimating epinephrin. When rabbit intestine segments were employed to check the results obtained on uterus segments, no epinephrin was detected in the venous blood either of normal men (17) or of patients suffering from various diseases, including Graves' disease (18).

The result of the other two experiments on emotional excitement (cats 4 and 5) was the same as in the first experiment.

Cat 4. Condensed protocol. Female. Weight, 2.0 kgm. The procedure was the same in cat 25. Bloods were obtained from a catheter as follows:

1. From adrenal level while cat was quiet.
2. From adrenal level after ten minutes of intense excitement (by dog).
3. With orifice of catheter withdrawn 7 to 8 cm., in lower part of cava.

Then obtained blood from the femoral artery through a cannula. As in the other experiments, the catheter was removed, cleaned and again oiled before collection of each specimen.

In this experiment some of the blood tests on the intestine segments were carried out in the usual way. Figure 16 shows that the "excited" blood did not cause any definite inhibition when it replaced "quiet" blood. For the sake of comparison, in other tests, the cylinder was emptied when a change of liquid was made (fig. 17). The apparent increase of tone in the segment at 9 is partly due to the mechanical effect of its weight coming on the lever when the Ringer's solution was removed. The same is true of the rise of the curve at 11, when the

"quiet" blood was withdrawn. The drop in the curve at 12 when the "excited" blood was introduced is likewise a purely mechanical effect, due to floating up of the segment. There is no inhibition. At 13 the blood was removed and the weight of the segment carried up the writing point. The drop at 14, when Ringer's solution was introduced into the cylinder, is quite decided, since not only was the segment floated up (mechanical effect), but the increase of tone was removed by the washing out of the blood by the Ringer's solution. There is no possibility of confounding the drop at 12 with a genuine inhibition, at least when the record begins with the segment initially beating in Ringer's solution, and not after blood has been applied to it, for the curve just regains the level it started with at 11. At 14 also there is no inhibition, the segment coming back to the same length which it had before 9.

The third experiment, on a female cat, was performed precisely like that on cat 4 and yielded a similar result.

SUMMARY

1. The relation of the epinephrin secretion of the adrenals to experimental hyperglycemias can be investigated under much better conditions in animals whose epinephrin output has been abolished or greatly reduced by removal of one adrenal and section of the nerves of the other, than in animals deprived of both adrenals. For in the first case the animals, after recovery from the operation, remain indefinitely in good health, whereas after total adrenalectomy observations on the blood-sugar are complicated by the fact that they must be made: *a*, practically on dying animals (unless survivors in species where accessory adrenals are common are employed) and *b*, on animals suffering from the immediate effects of a major operation and anesthetization.

2. The hyperglycemia associated with asphyxia and ether anesthesia is obtained in cats which have undergone the adrenal operation described, even when no detectable residual liberation of epinephrin is present. No essential difference could be made out in this regard between these animals and control normal cats.

3. Accordingly, the mobilization of sugar, of which these experimental hyperglycemias are the expression, is not mediated through the epinephrin secretion of the adrenals.

4. Such observations as we have made on the effect of fright do not support the view that so-called emotional hyperglycemia is a constant

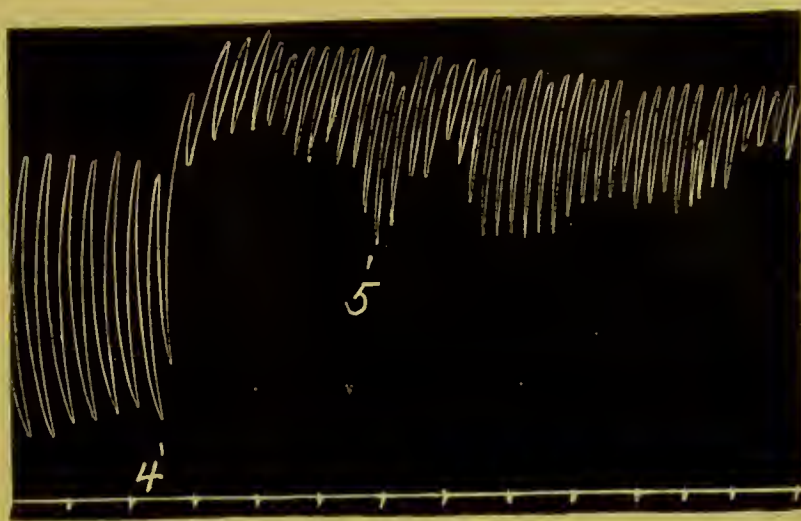


Fig. 16. Intestine tracings. Bloods from cat 4. At 4 Ringer's solution was replaced by the first catheter specimen obtained from the adrenal level while the cat was quiet. At 5, this was replaced by the second catheter specimen from the same level after the cat had been excited for ten minutes. (Reduced to one-half.)

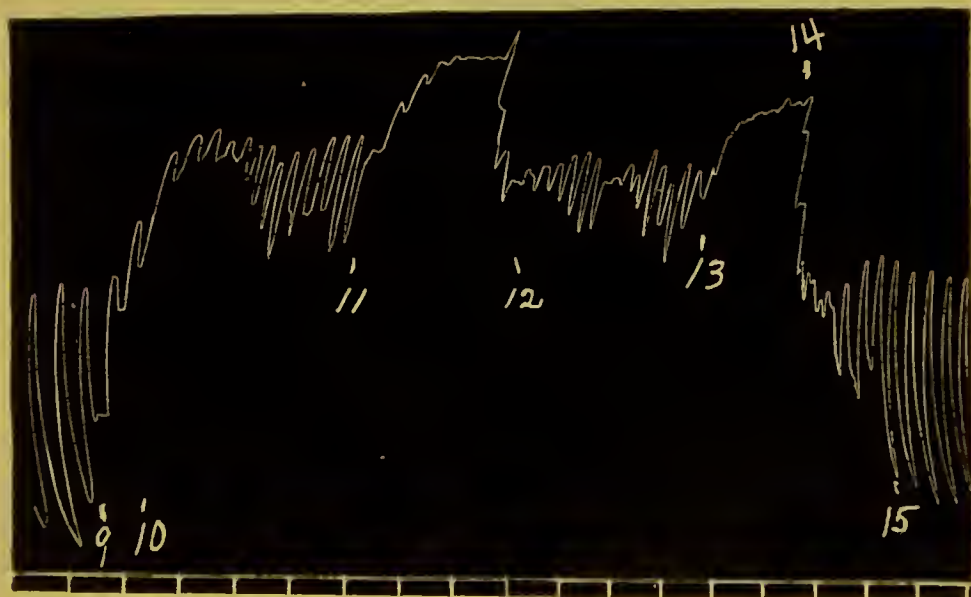


Fig. 17. Intestine tracings. Bloods from the same cat used for figure 16. At 9 the Ringer's solution was removed from the cylinder; at 10 the first catheter specimen of blood (quiet) was introduced; at 11 the blood was removed; at 12 the second catheter specimen (excitement) was introduced; at 13 the blood was removed; at 14 Ringer's solution was introduced; at 15, the Ringer's solution in the cylinder was displaced by the introduction of Ringer's solution from a fine pipette, with its orifice at the bottom of the cylinder. (Reduced to one-half.)

or even a common phenomenon in cats. If it exists, it does not depend upon an increase in the epinephrin liberated from the adrenals. For *a*, no essential difference could be detected between the results of emotional disturbance on the blood-sugar content in the cats whose epinephrin output had been interfered with, and in the control normal cats; *b*, no evidence was obtained that emotional disturbance increases the output of epinephrin in normal cats.

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ATTEMPTS TO PRODUCE A SUBSTANCE WITH THYROID-LIKE ACTIVITY BY THE ARTIFICIAL IODIZATION OF PROTEINS

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It has been shown by Morse (1) and confirmed by us (2) that artificially iodized blood serum (Iodalbin, Parke, Davis and Company), when fed to tadpoles, causes acceleration of metamorphosis similar to the effects of feeding with desiccated thyroid, although not as rapidly nor as marked, in comparison with the amounts of iodine present. We have attempted to determine whether such activity can be demonstrated after artificial iodization of the component protein fractions of blood serum obtained by the crude albumen-globulin separation resulting from salting the serum with ammonium sulphate, and also if proteins other than those represented by blood serum showed this activity when iodized. In addition, we have investigated whether such activity can be increased by concentration of the products, by the method of alkaline hydrolysis employed by Kendall (3) in concentrating the active principle of the thyroid.

Employing the method described by Kurajeff (4) we have artificially iodized whole ox serum, ox serum globulin (obtained by half-saturation of the serum with ammonium sulphate), ox serum albumen (obtained by saturating the filtrate of the globulin with ammonium sulphate), Merk's egg albumen and egg white (obtained from fresh eggs). We also used commercial iodized blood protein (Iodalbin) and Iodocasein (Mulford). From all of these iodized proteins we also prepared products "A" (Kendall) by alkaline hydrolysis.

The iodine contents of these preparations are given in table I. The artificially iodized proteins and their products "A" show wide variations in their iodine contents. No particular significance is attached to these differences, since no attempt was made to completely remove the free iodine.

The plan of feeding was the same as described in our previous work, in which the tadpole reaction was employed. All experiments were carried out in duplicate. The tadpoles received liver every other day and a dose of the substance to be tested on the alternating days. The tap water in the dishes was changed twice daily.

Each of the iodized proteins was offered in doses of 1, 2 and 5 mgm. and their products "A" in doses of 0.5, 1 and 2 mgm.

Product "A" of fat-free hog thyroid was fed to one set of tadpoles and the activity observed for comparison with the other preparations. This product, as well as the desiccated thyroid from which it was obtained, had been used in our previous investigations and our familiarity with its activity led us to employ it for comparison with these substances.

Two series were studied, one on young tadpoles (started May 28 and about twelve days old) and another on older ones (started June 20 and about twenty-five days old). We have previously pointed out that the most reliable results are obtained with older tadpoles (2 and 5). The results obtained are given in condensed form in table 1. Our chief desire in the present investigations being to ascertain whether any of the preparations demonstrate a distinct action, we have made no attempt to determine their quantitative activities, and the results in the table represent the composite activity of all doses administered. Very marked activity is indicated by I, marked activity II, moderate III, weak IV, very weak V, doubtful VI, and normal growth (as shown by the controls) VII.

A striking difference between the iodized proteins and thyroid was indicated from the results obtained by feeding their products "A" to tadpoles. Alkaline hydrolysis of the thyroid always concentrates the active iodine yielding the very active product "A" while with the iodized proteins it is apparent that

the process of hydrolysis disrupted the active iodine combination, so that the resulting product "A" gave practically no effect. This leads to the conclusion that the iodine complex in thyroid, being in a much more stable combination, is not identical with that of the iodized proteins. The very slight activity, observed in a few instances, of the products "A" can be explained by the fact that the crude process of hydrolysis may have been more or less incomplete with some of the preparations, and the separation of the product "A" from the iodized

TABLE 1

IODIZED PROTEIN	IODINE CONTENT	ACTIVITY SERIES 1	ACTIVITY SERIES 2	IODINE CONTENT OF PRODUCT "A"	ACTIVITY OF PRODUCT "A" SERIES 1	ACTIVITY OF PRODUCT "A" SERIES 2
	<i>per cent</i>			<i>per cent</i>		
Iodized ox serum . . .	11.69	III-IV	II-III	6.15	IV	V-VI
Iodized ox globulin . .	20.3	II-III	I-II	20.3	VI	VI
Iodized ox albumen . .	20.3	III-IV	III	17.53	V-VI	VI
Iodalbin (Parke, Davis & Co.)*	21.5	IV	IV	6.15	V	V-VI
Iodocasein (Mulford)	18.0	IV	III-IV	6.76	V	V-VI
Iodized egg albumen (Merk)	12.3	V	IV-V	12.92	VI	V-VI
Iodized fresh egg white	11.69	V	III-IV	14.15	VI	V-VI
Fat free hog thyroid .	0.21			1.02	I	I
Controls		VII	VII		VII	VII

* Activity was distinctly lower than a specimen used in another series of experiments.

protein also being crude, the product may contain a small quantity of the material from which it is derived, which is still unchanged.

The iodized egg protein caused a very little effect, that prepared from fresh egg white being somewhat more active than the preparation made from commercial egg albumen (Merk's).

Casein being an isolated protein, it is of interest to note that when iodized (Iodocasein) it is capable of showing activity on tadpoles. This is in harmony with the accepted view that the active iodine complex of the thyroid is in combination with

tyrosine or tryptophane, casein being relatively rich in both these amino acids (tyrosine 4.5 per cent and tryptophane 1.5 per cent), to which its activity when iodized may be due. In this regard it is interesting to note that egg albumen is relatively poorer in tyrosine (1.1 per cent) than either casein, serum-albumen (2.1 per cent), or serum-globulin (2.5 per cent).

In these experiments, we found Iodalbin to show distinct activity, but much less than another specimen of Iodalbin showed in another set of experiments. This product showed distinctly less activity on the tadpoles than the iodized blood proteins prepared by us, while the specimen used in our other set of experiments was fully as active. This can possibly be explained on the supposition, that different specimens of blood vary in their contents of the necessary nucleus with which the iodine must combine.

It appears from the results obtained by us with the tadpole reaction, that this nucleus is most abundant in the globulin fraction of the blood serum. It will be seen that the greatest activity displayed by any of the artificially iodized proteins was shown by the serum globulin. The albumen fraction was somewhat less active than the whole serum. Allowing for the crudeness of the separation, the indications are that the globulin fraction represents most of the activity represented by iodized blood serum. The fact that different sera when iodized show differences in activities also indicates that the amount of the necessary nucleus present varies in the different bloods.

The striking activity exhibited by the iodized ox serum globulin is a very interesting observation, in as much as the active iodine of the thyroid is known to be in combination with the thyroid globulin. It is indeed possible that in the animal organism, iodine is carried to the thyroid in combination with the serum globulin and is then converted into the thyroid hormone by further action contributed by something (probably an enzyme) in the thyroid. The probability of this hypothesis is strengthened by the fact that no activity can be elicited from hyperplastic thyroids or their concentrated product "A" when artificially iodized (2) and also by the fact that alkaline hydrolysis destroys the activity of iodized serum globulin, therein

differing from the activity of the thyroid, which may indicate that the globulin of the blood contains the necessary nucleus (amino acid groups) with which the iodine must combine, and the thyroid supplies the activating substance (enzyme?) both of which must come into contact with each other to complete the stable iodine combination or hormone which is active in the thyroid and is concentrated by alkaline hydrolysis. More information on this point might be revealed by investigating the activity of iodized sera obtained from goiterous animals and from thyroidectomized animals, which we had hoped to study in the near future, but owing to the fact that one of us (Dr. Marine) has been assigned to military duty, the progress of this investigation has been interrupted and we have deemed it advisable to report the results obtained by us up to the present.

SUMMARY

1. Artificially iodized blood serum (ox) causes acceleration of metamorphosis when fed to tadpoles, resembling that shown by thyroid, but not so marked nor so rapid.
2. It is possible to show by crude process of separation that the globulin fraction of the serum contains most of the substance with which the iodine combines to give the activity on tadpoles.
3. Alkaline hydrolysis of iodized proteins apparently destroys their activity, therein differing from thyroid. This suggests that the thyroid adds something to the iodine complex in the blood to complete the stable iodine containing thyroid hormone.

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